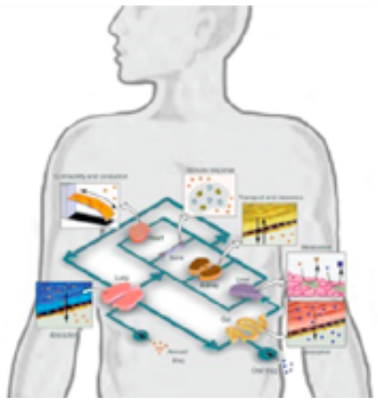
THIRD WORKSHOP ON VALIDATION AND QUALIFICATION OF

NEW IN VITRO TOOLS AND MODELS FOR THE PRE-CLINICAL DRUG DISCOVERY PROCESS

MARCH 14-15, 2013 | LISTER HILL AUDITORIUM, NIH CAMPUS | BETHESDA, MD



Disease-Specific Integrated Microphysiological Analysis Platforms (iMAPs)

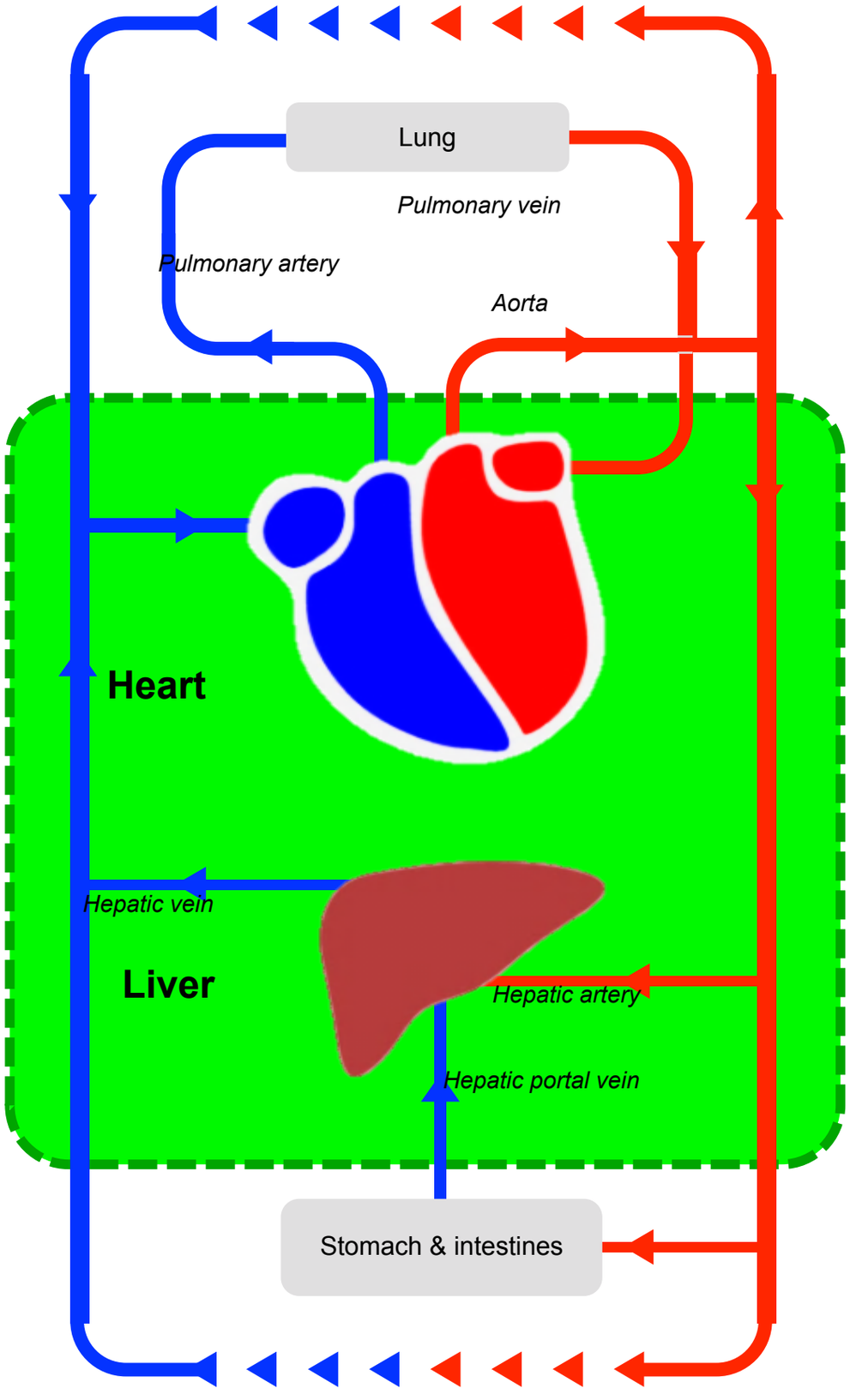
Kevin E. Healy & Luke P. Lee

UC Berkeley

Holger Willenbring & Bruce Conklin

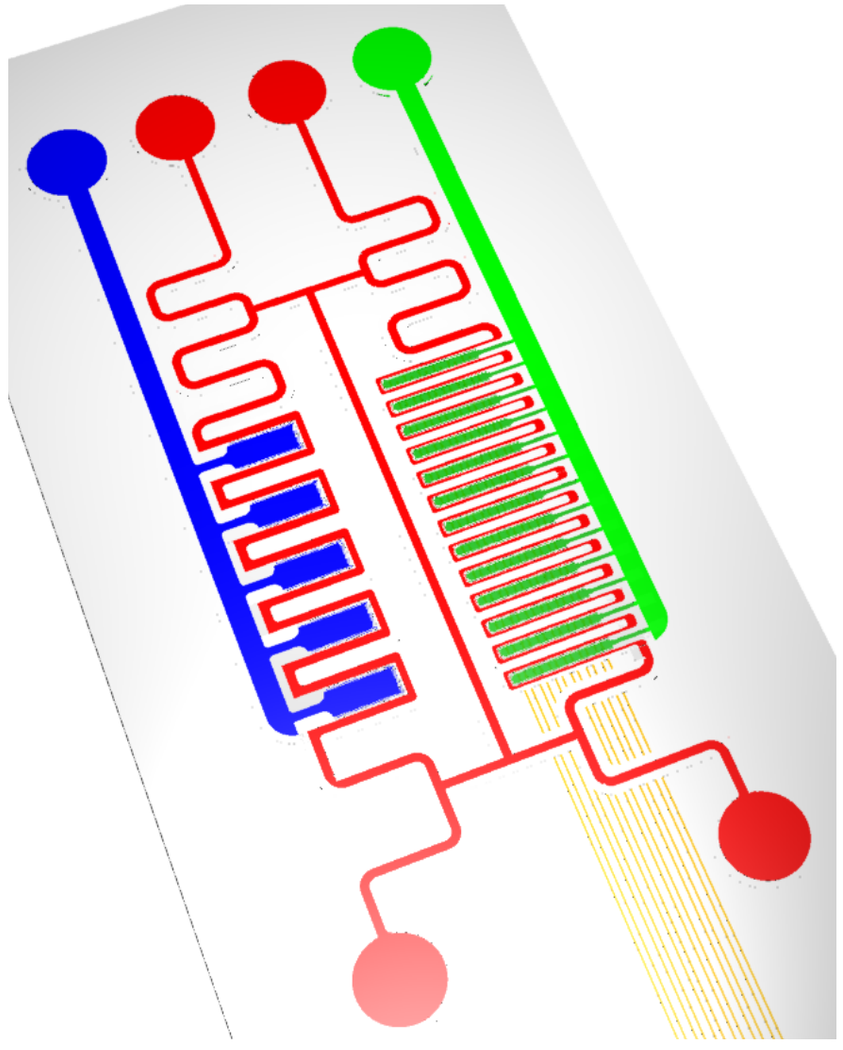
UCSF & Gladstone Inst. Cardiovascular Disease

Disease-Specific Integrated Microphysiological Human Tissue Models



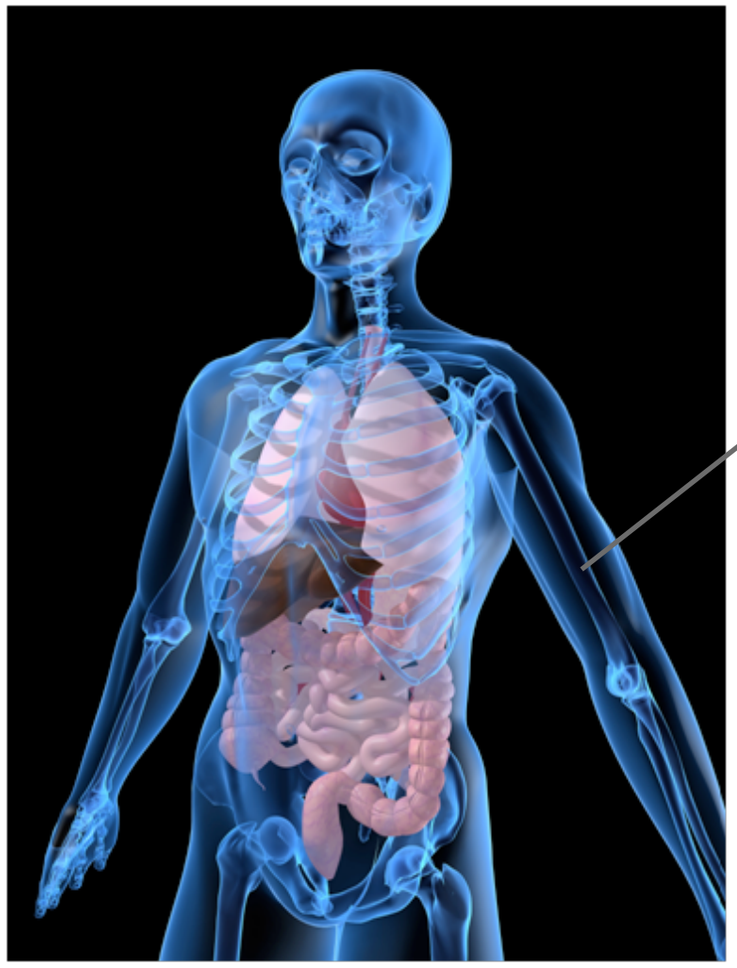
Most drugs fail due to cardiac or liver toxicity

Integrated *in vitro* models of human cardiac and liver tissue

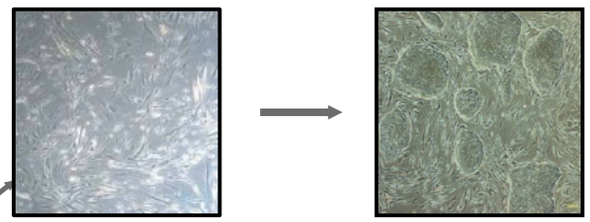


Patient-Specific integrated Microphysiological Analysis Platforms (*iMAPs*)

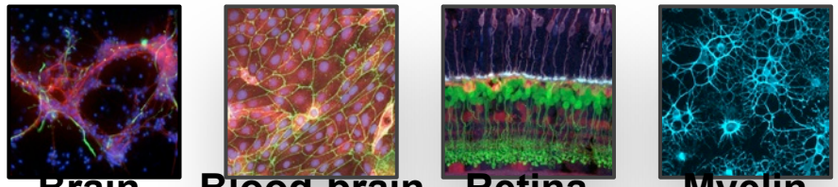
Human iMAPS

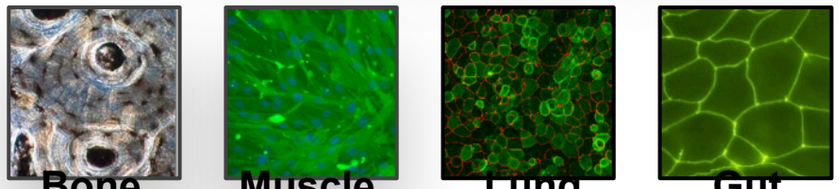


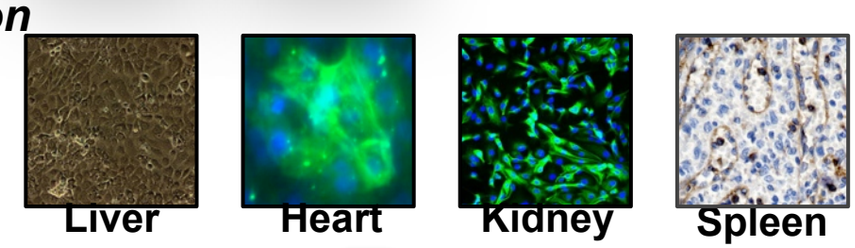
Somatic cell **Patient-hiPSC**

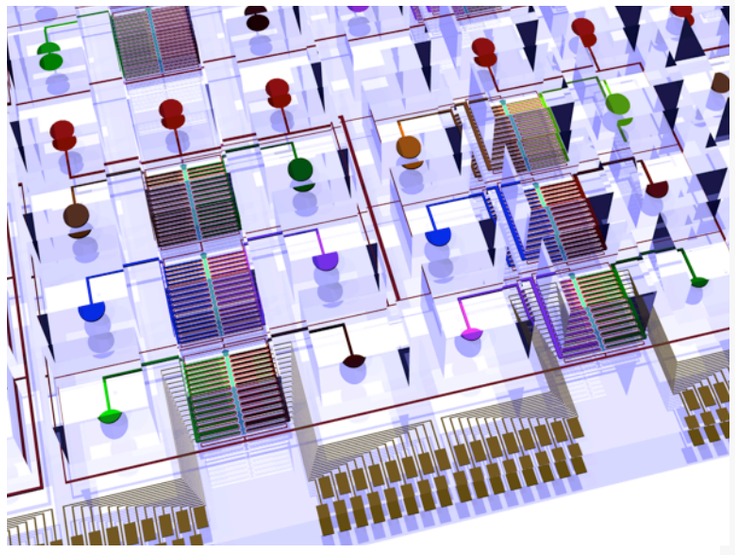


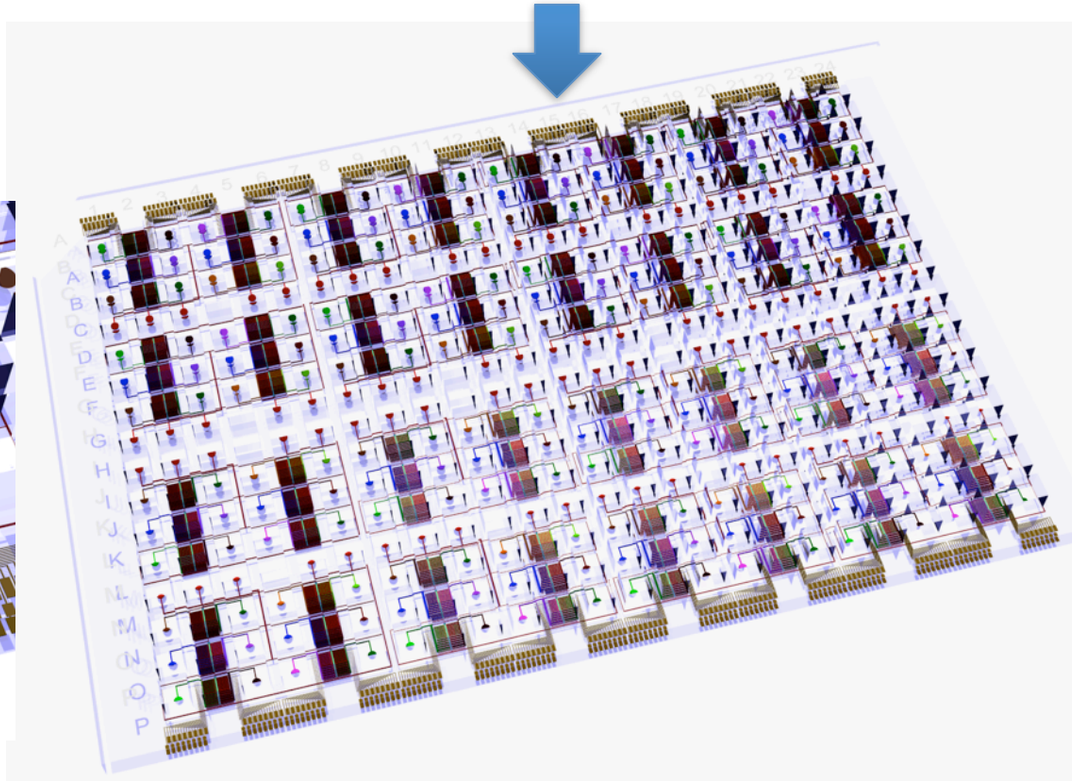
Reprogramming ***Differentiation***





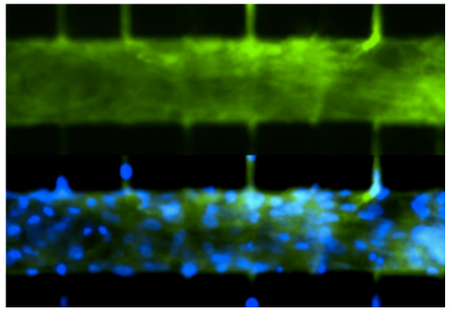




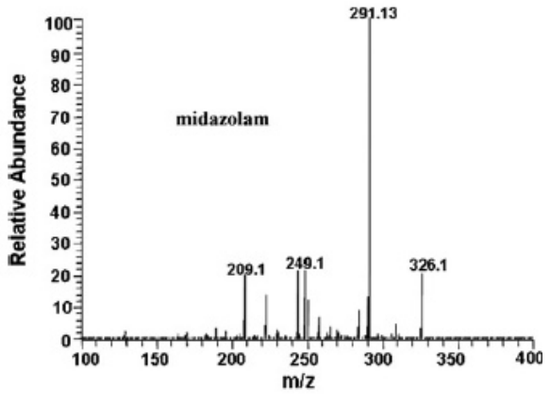


Patient-Specific integrated Microphysiological Analysis Platforms (*iMAPs*)

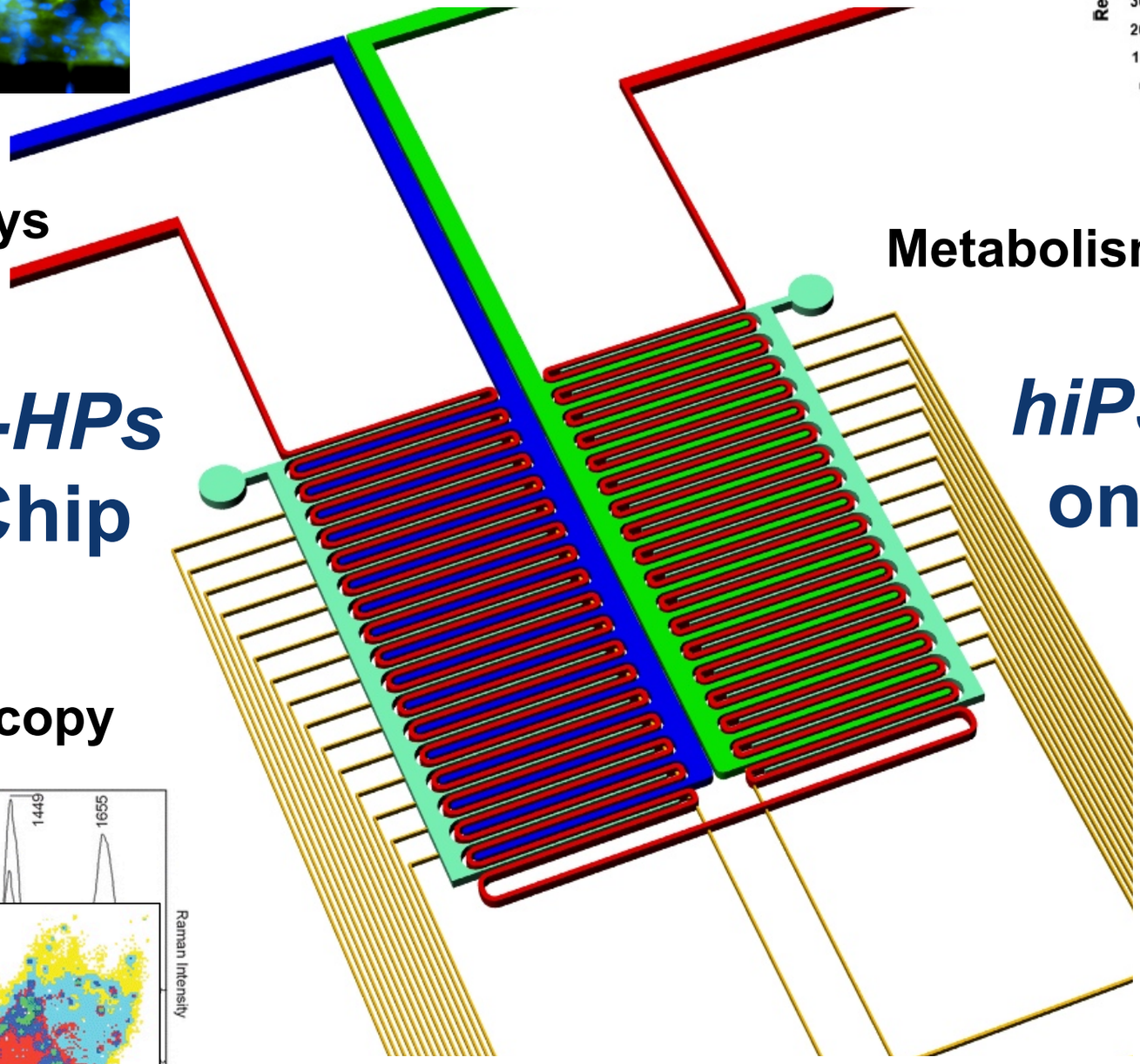
Optical Microscopy



Mass Spectrometry



Real Time Sampling



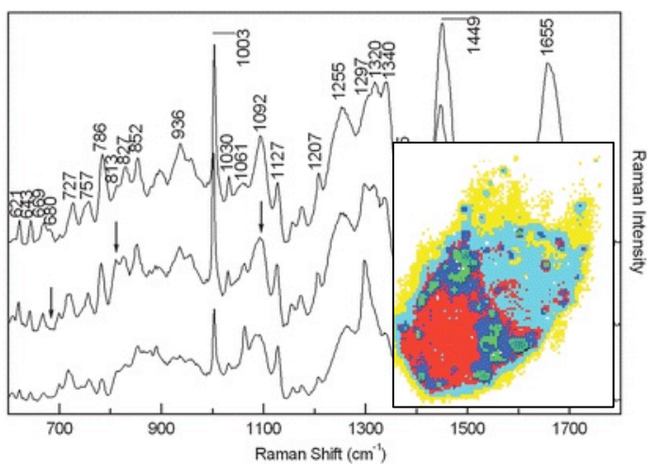
Elisa & mAb Arrays

Metabolism - SeahorseBio

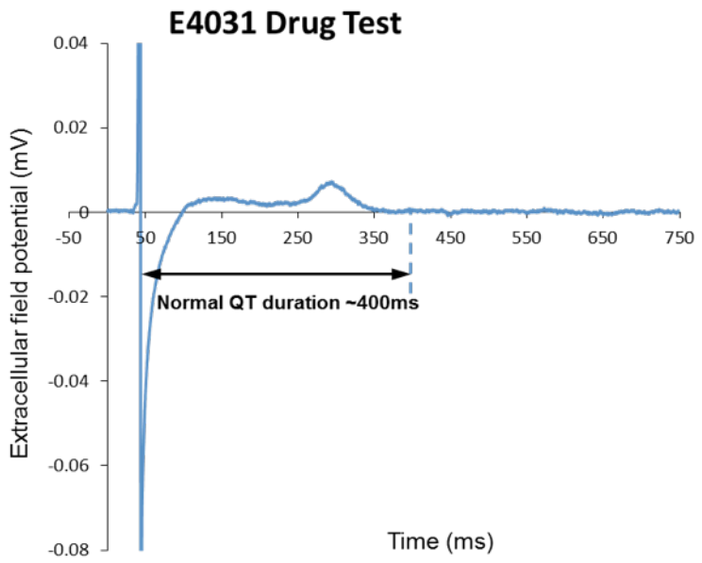
hiPS-HPs on Chip

hiPS-CMs on Chip

Raman Microscopy



Electrophysiology

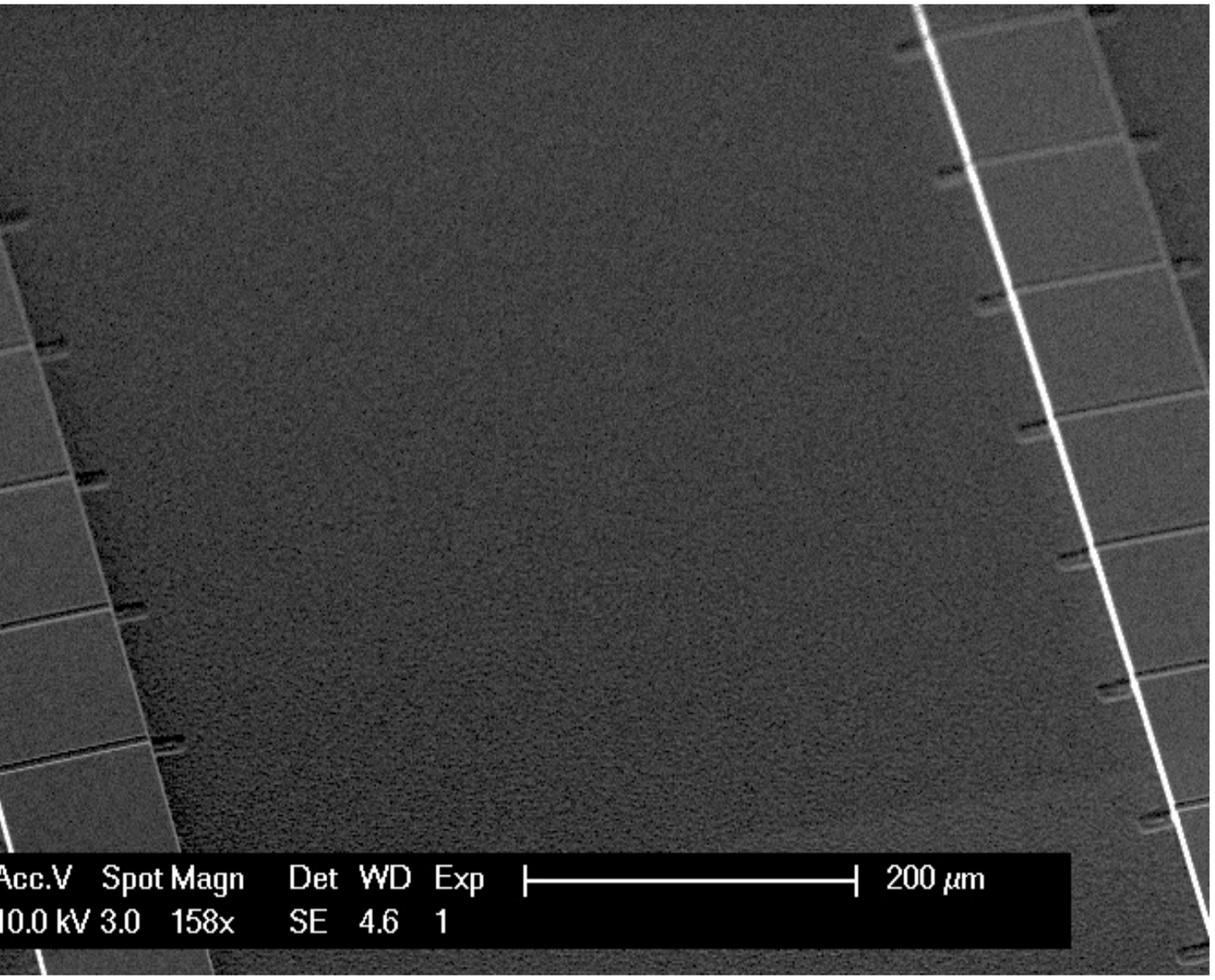


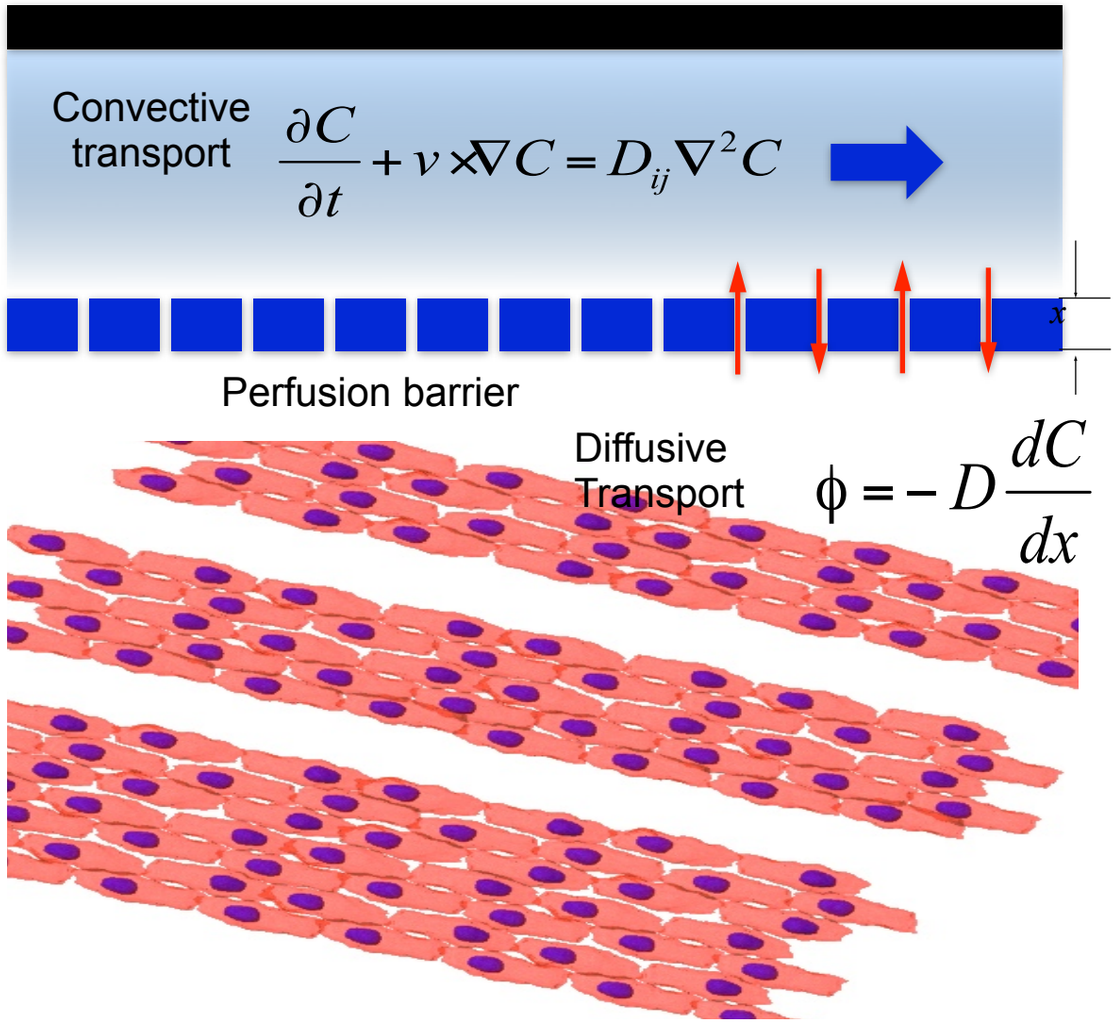
Destructive Sampling

qPCR, RNAseq

Physiologically Relevant Precision Biology by Biomimetic Cell Culture

What is the minimal organ size or organoid to assist in drug discovery?





Cultured Cells

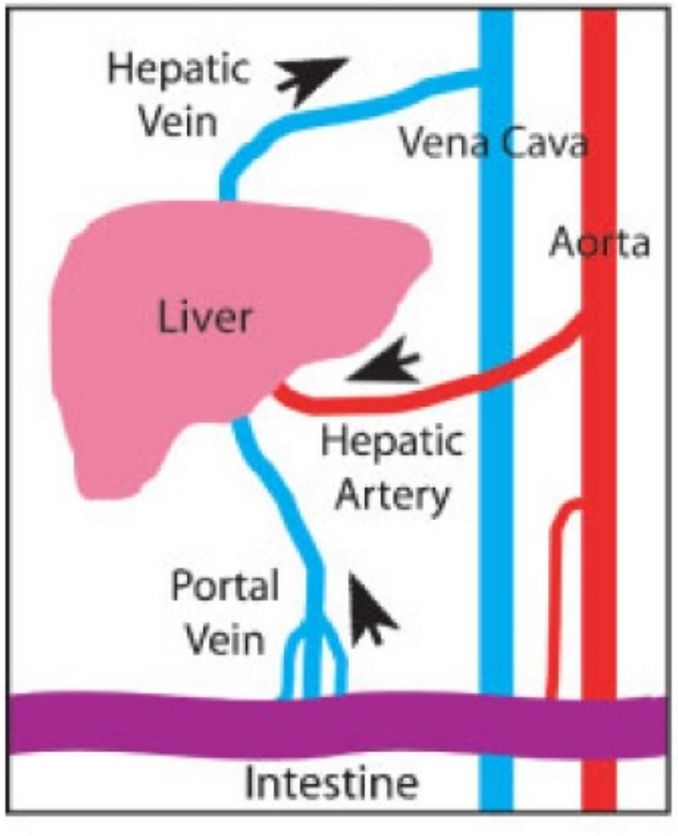
- Understand physiologically relevant microenvironments
- Use precision microengineering to create better cell environments
- Precision biological perturbations, real time and continuous monitoring

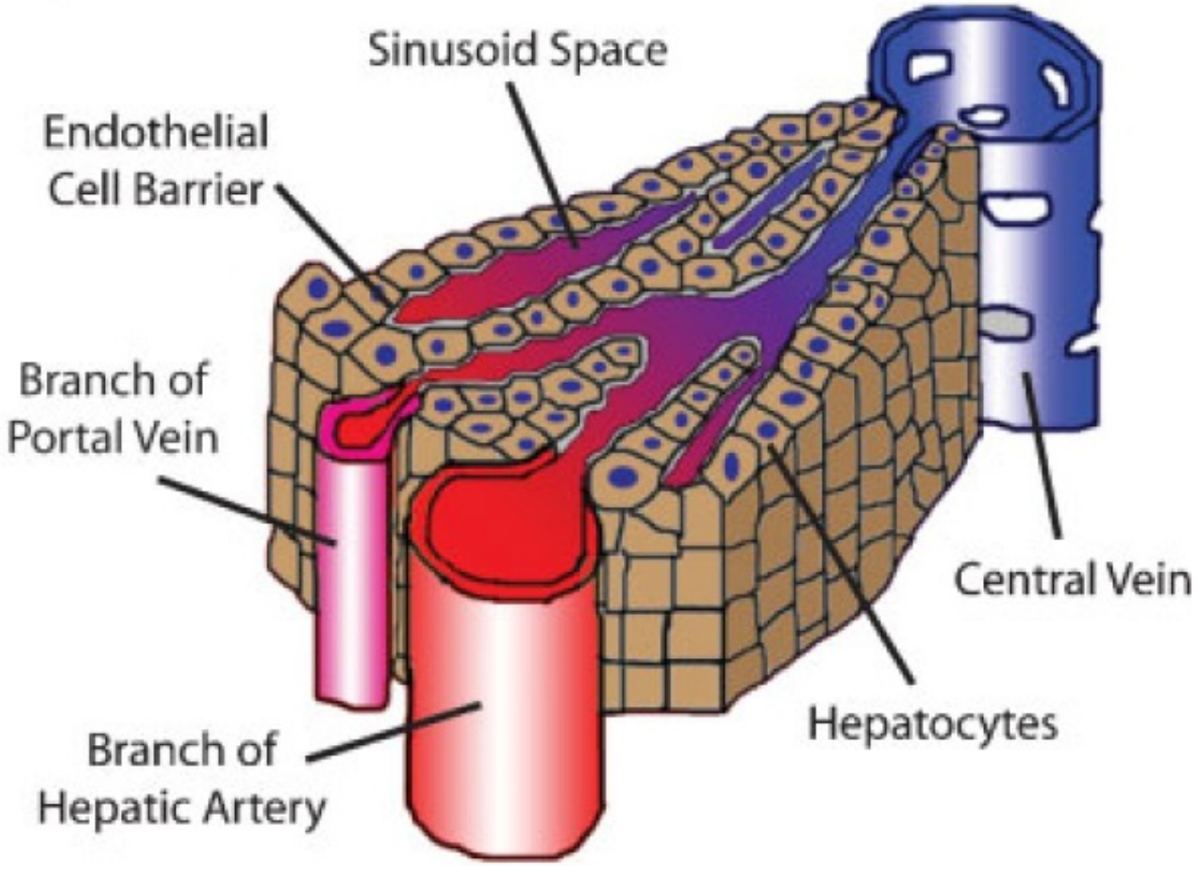
Milestones - Year 1

Milestone 4. To generate a population of normal hiPSC-derived HPs that resembles primary human hepatocytes in drug metabolism activities.

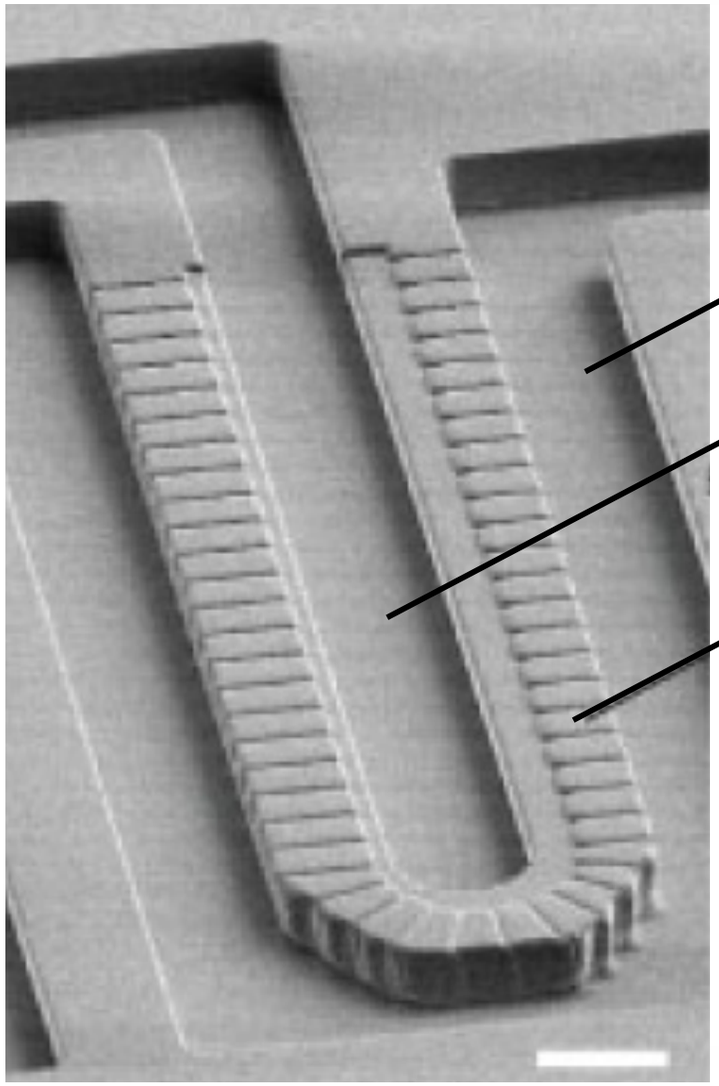
Goals and timeline: To identify hiPS cell lines from clinically normal donors that produce HPs that have drug metabolism activities similar to normal hpHPs. 18 months.

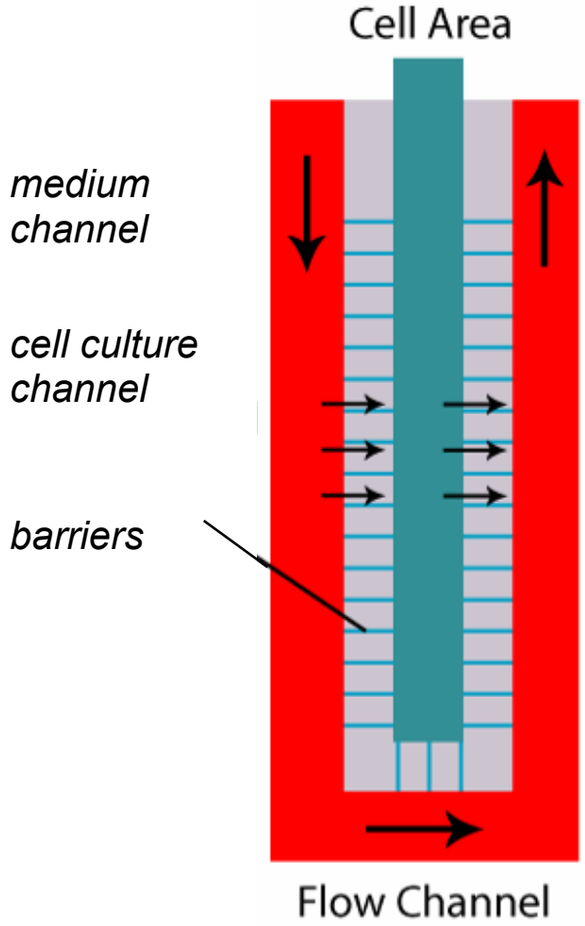
Criteria for Success: Generation of hiPSC-derived HPs that exhibit drug metabolism activities similar to freshly isolated hpHPs. Achieving this milestone will require the following: (1) The purity of the hiPSC-derived HPs will be at least 80% as measured by FACS for HP-specific markers. (2) The activities of enzymes and transporters critical for hepatic drug metabolism will be at least 50% of those of hpHPs. Since the cytochrome P450 enzymes are critical for phase I drug metabolism as well as general metabolism of the human liver, the characteristics of the hiPSC-derived HPs will be assessed by the quantitative measurements of the activities of CYP1A1, CYP2B6, CYP2Cs, CYP2D6 and CYP3A4. In addition, the activities of 2 phase II (UGT and SULT) drug-metabolizing enzymes, 3 phase 0 uptake transporters (OATP1Bs, OATs, OCTs), and 2 phase III efflux transporters (P-gp and BCRP) will be measured.

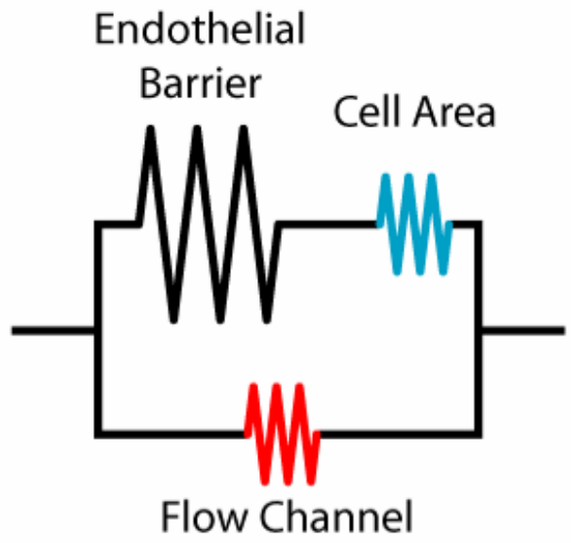




Model Liver Sinusoid



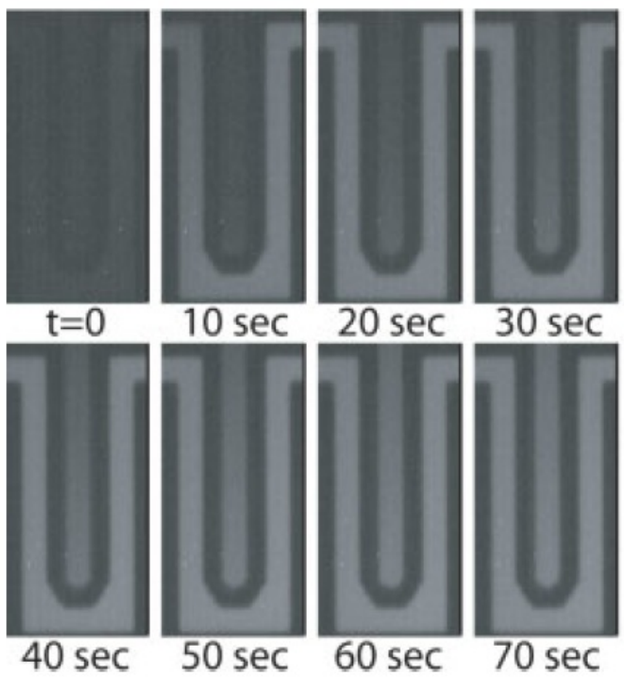


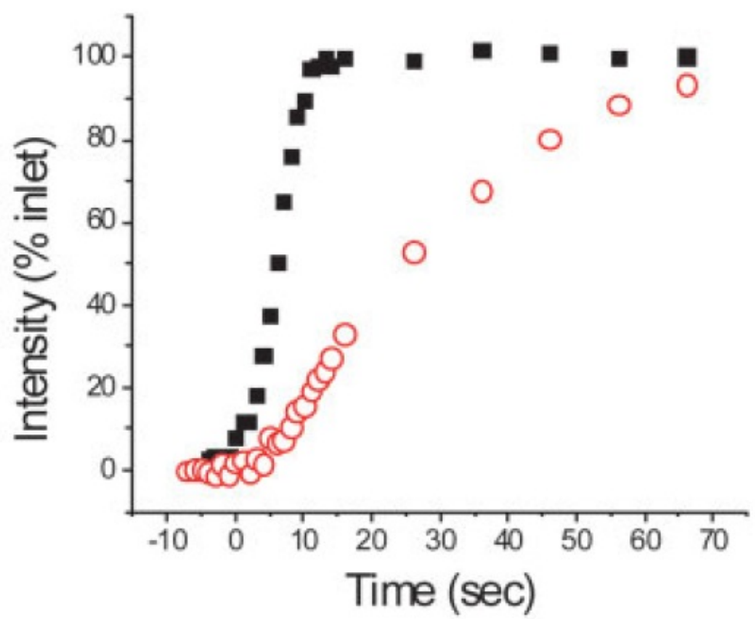


$$R_{hyd} \approx \frac{8\eta L}{\pi (d_h)^4}$$

$$d_h = \frac{4dh}{2d + 2h}$$

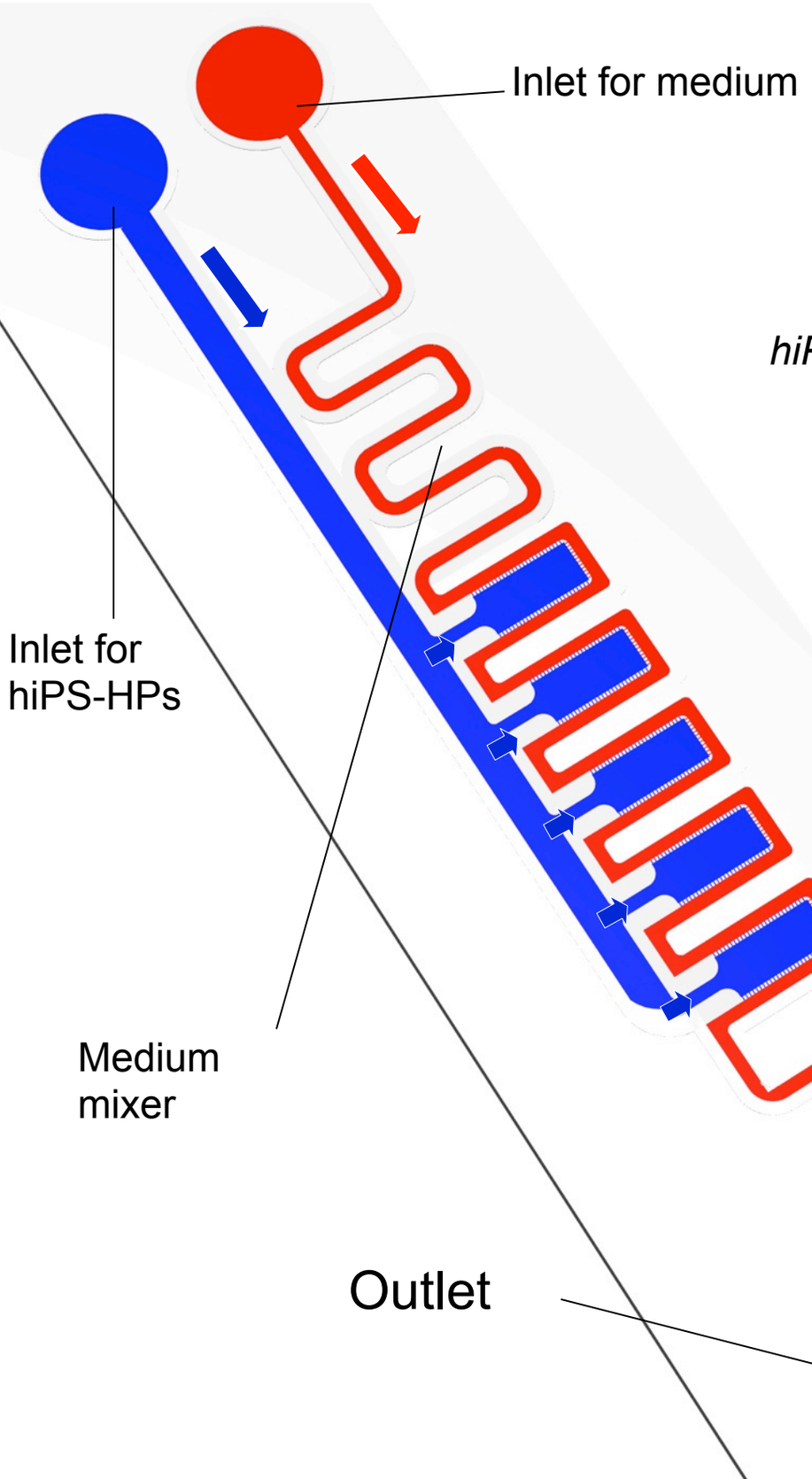
- Microfluidic endothelial-like cell barrier
- High density hepatocyte culture
- Continuous flow mass transport





Lee, P. et al., Biotechnol. Bioeng., 97, 1340–1346 (2007)

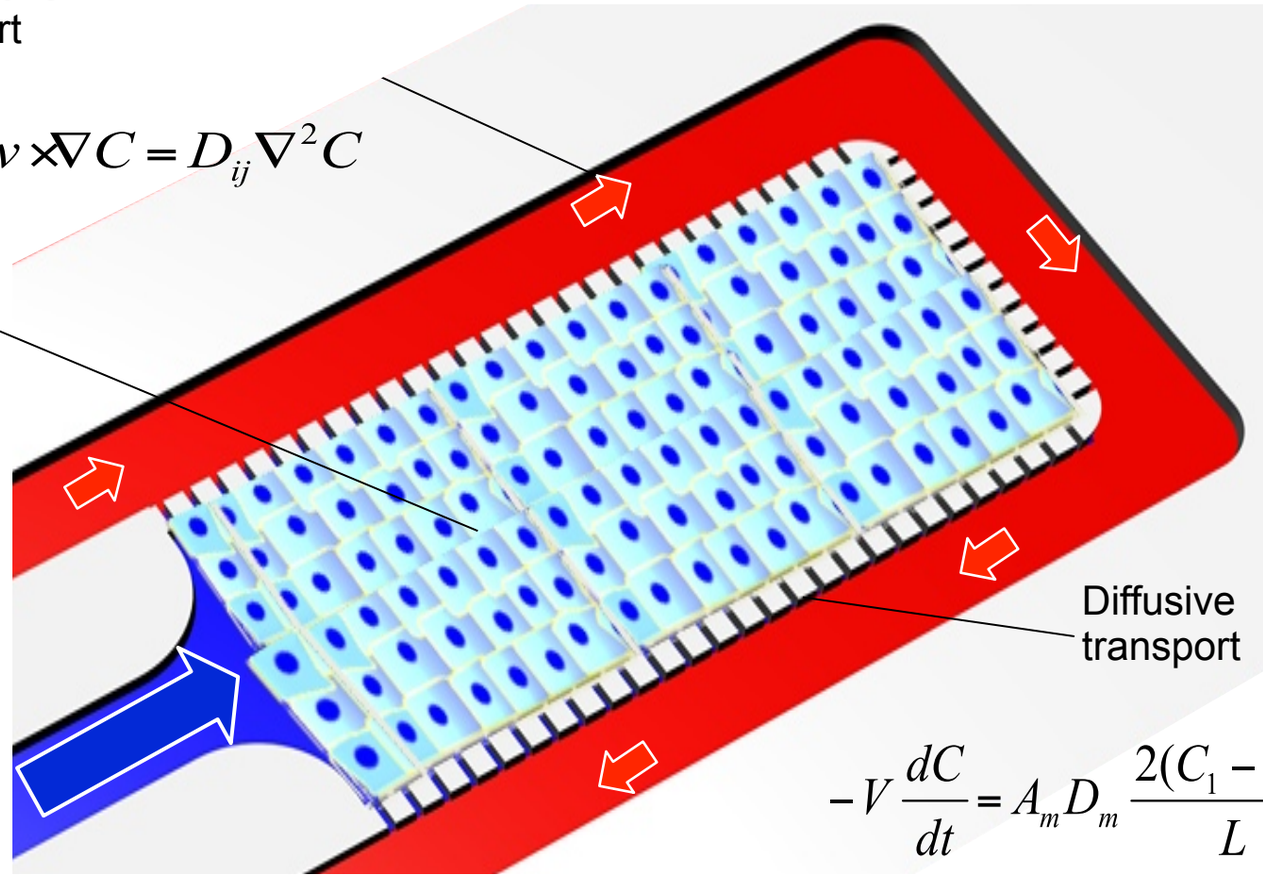
Disease-Specific Human Liver Tissue Model



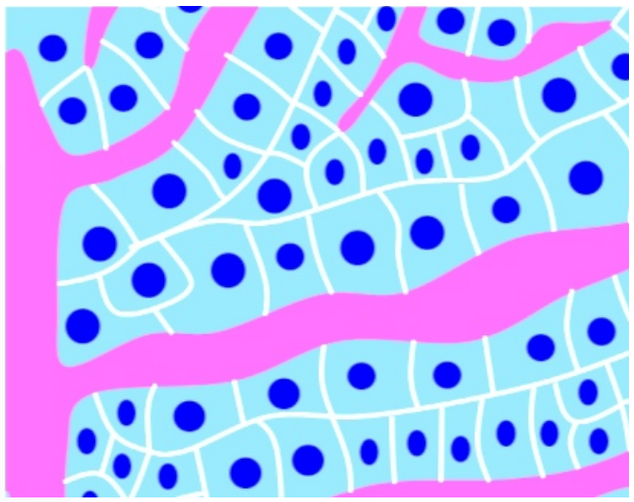
Convective transport

$$\frac{\partial C}{\partial t} + v \times \nabla C = D_{ij} \nabla^2 C$$

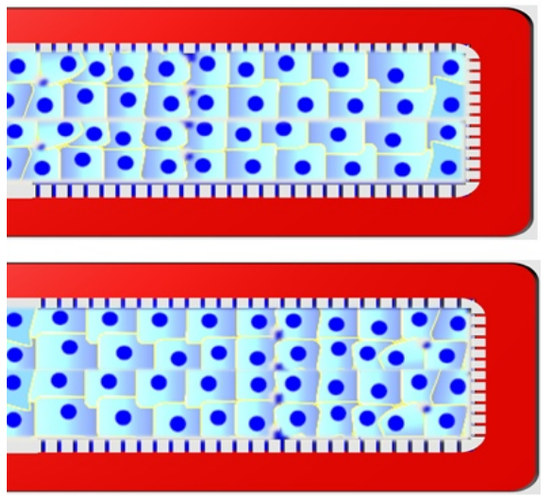
hiPS-HPs



$$-V \frac{dC}{dt} = A_m D_m \frac{2(C_1 - C_0)}{L}$$

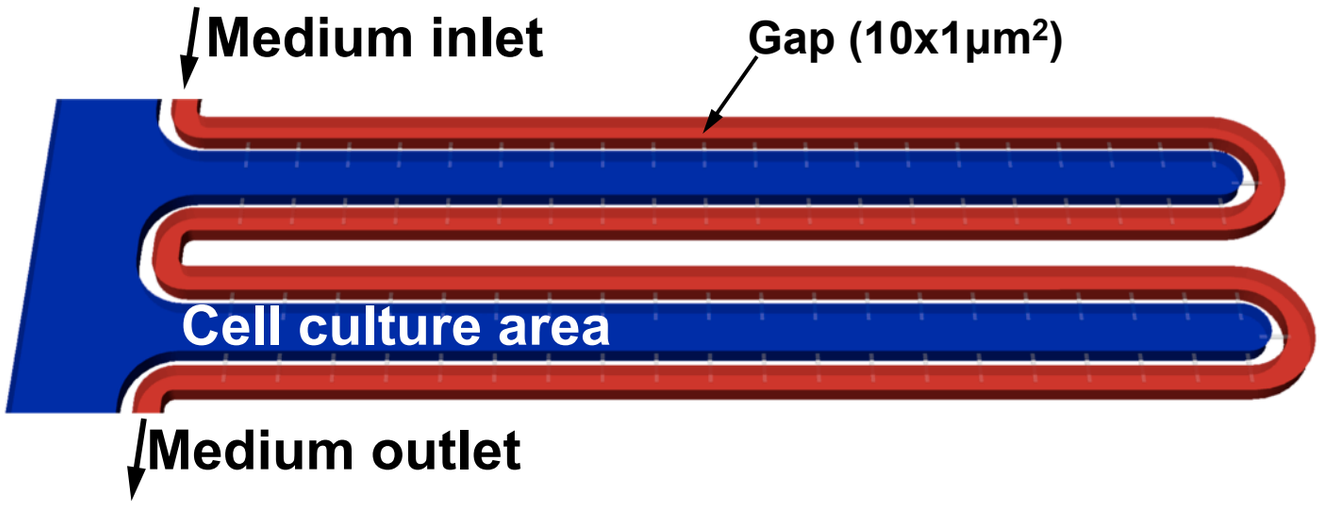


HPs in the liver



hiPS-HPs on-a-chip

Numerical Simulation: *Nutrient Profile*

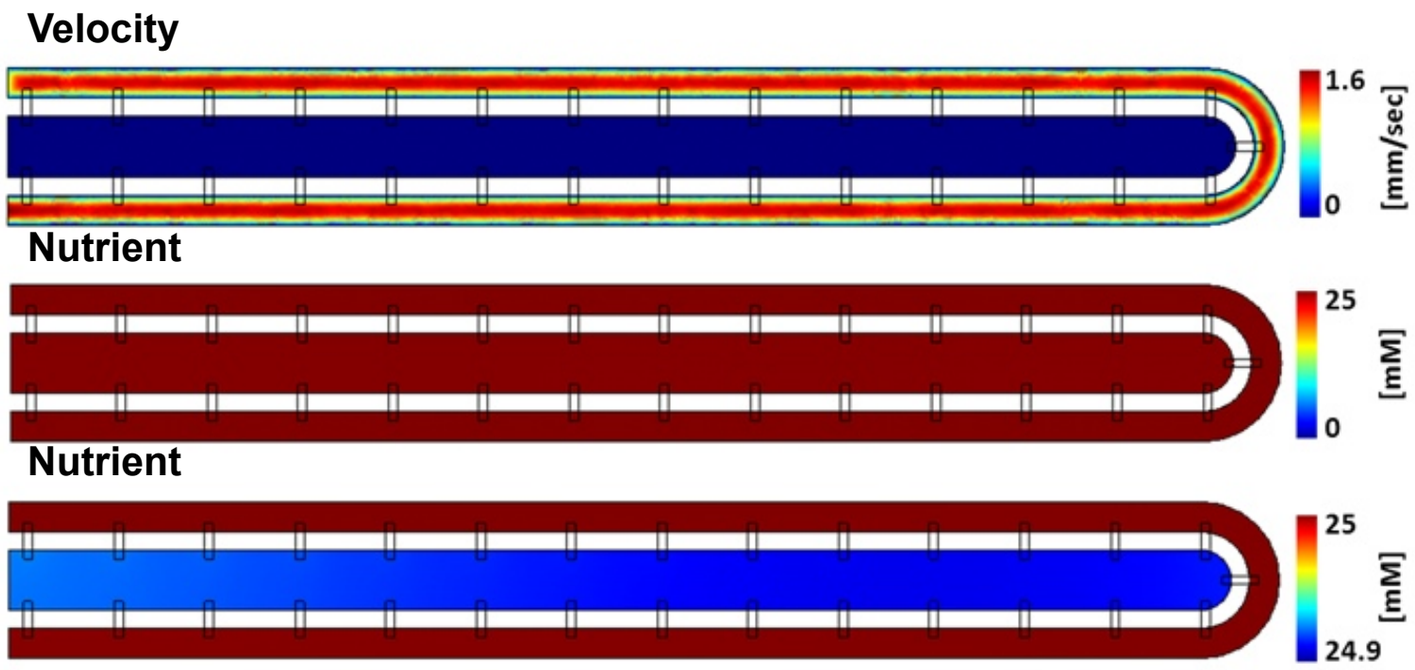


Governing equation:

$$-D\nabla^2 c = R - u \times \nabla c$$

D: Diffusion coefficient, ***c***: Concentration,
R: Reaction, ***u***: Velocity

Nutrient Profile



Simulation conditions

Nutrient concentration: 25mM

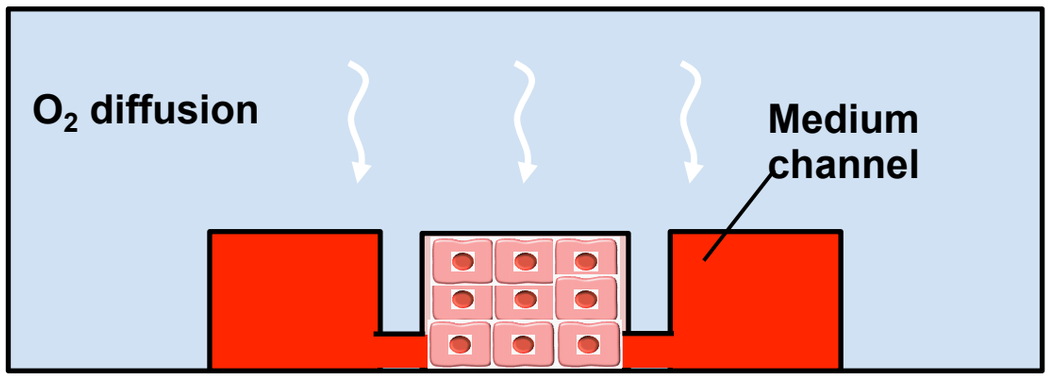
Cell number in the culture chamber: 2000 cells

Nutrient consumption rate: 10~40 fmol/cell day

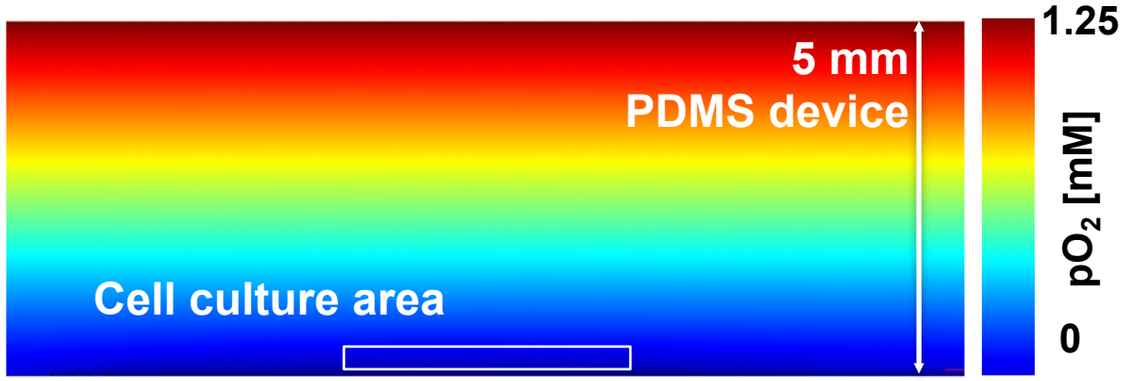
Flow rate: 0.1 μl/min (0.83 mm/sec)

Numerical Simulation: *Oxygen Profile*

Oxygen Gradients

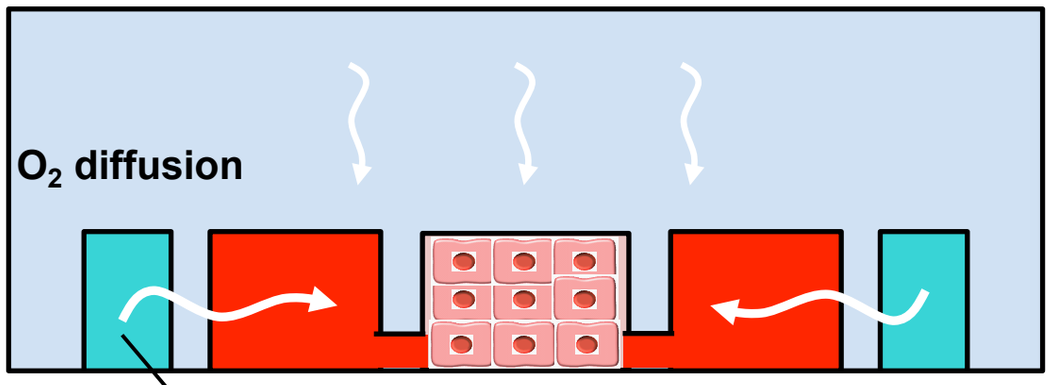


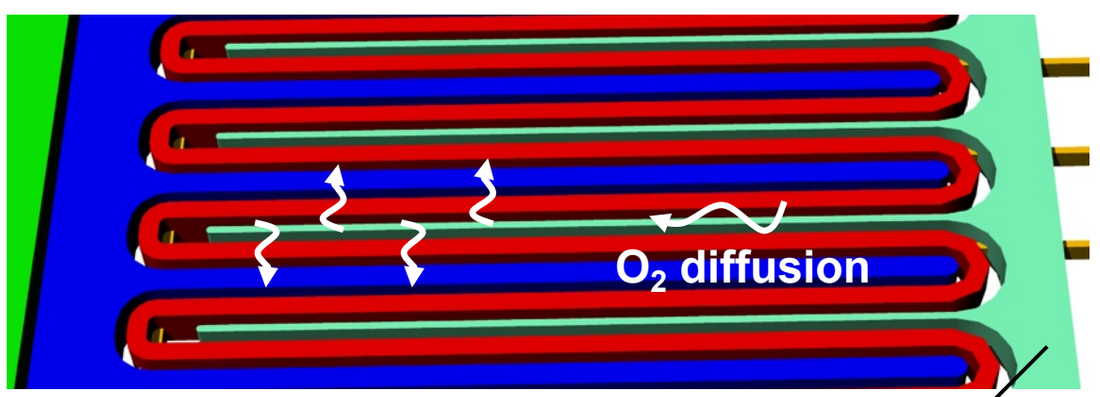
O_2 Profile



O_2 consumption rate: *3.8E-16 mol·cell^{-1}·day^{-1}*

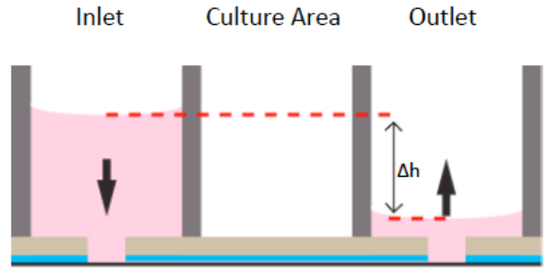
Solution: Air Diffusion Channel

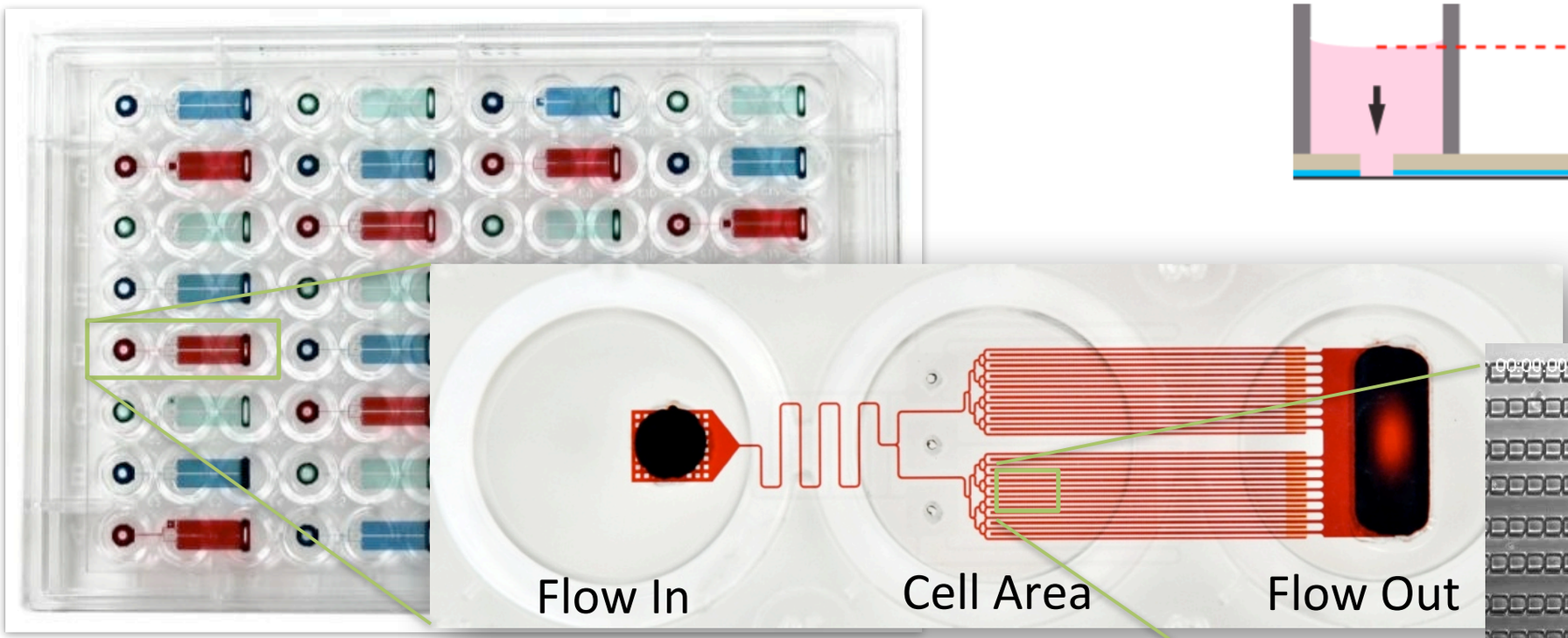


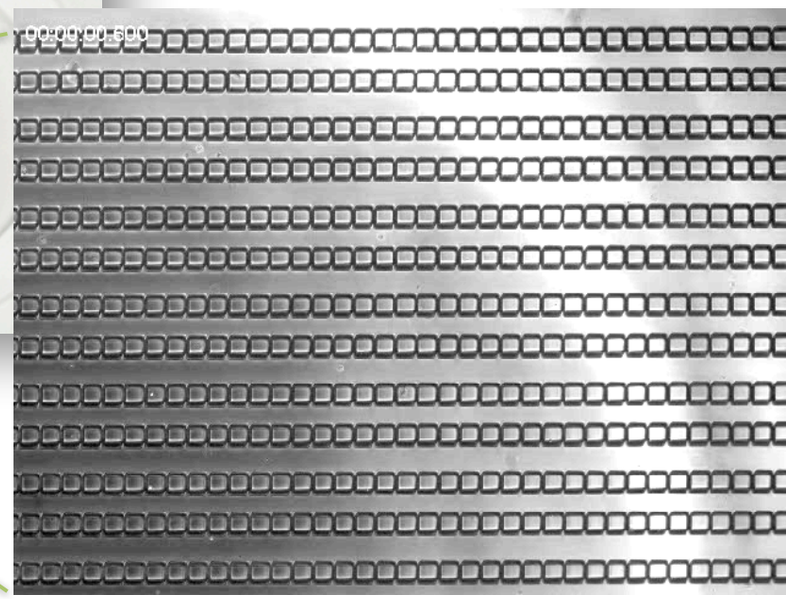


Microfluidic Liver Tissue Model Array

User-friendly Tubeless Microfluidic Interface







- ***Three well positions per flow unit***
- ***20,000-50,000 cells per culture unit***
- ***Continuous media flow (100 µl/day)***
- ***96 well format (32 flow units)***
- ***Gas exchange through permeable membrane***

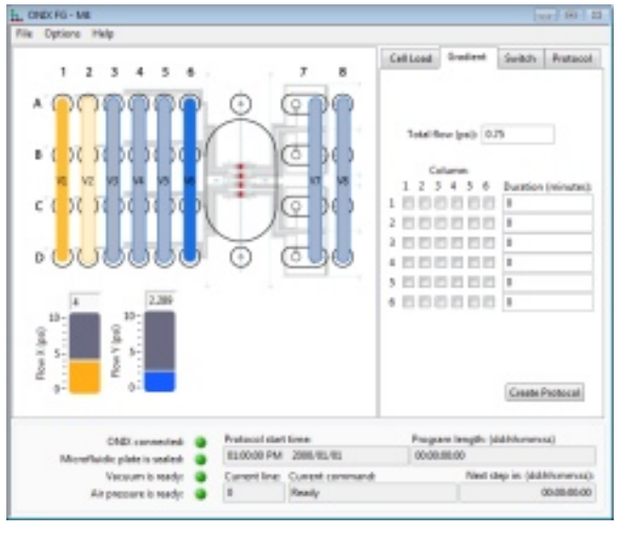
CellASIC

Microfluidic Liver Tissue Model Array

Control System

Manifold

Microfluidic Chip



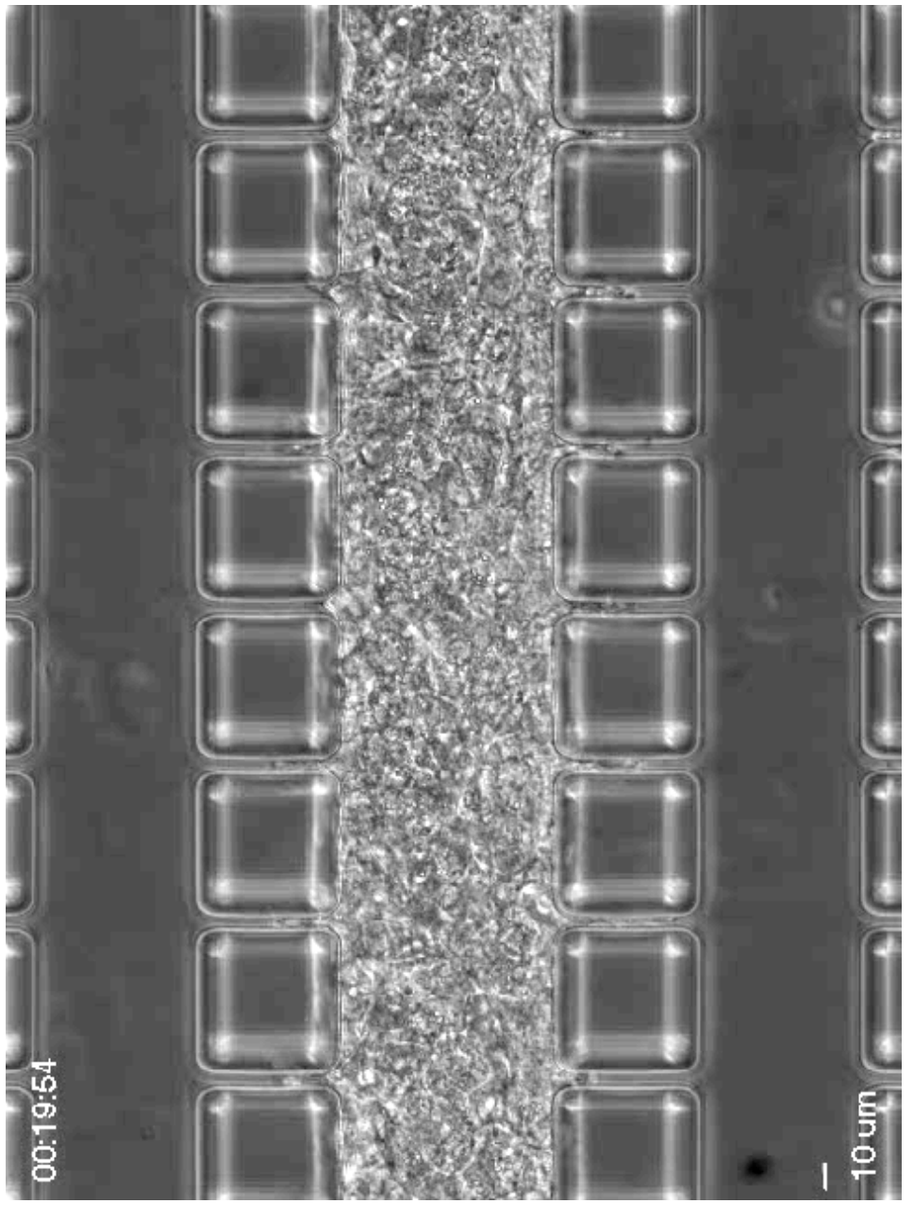
Software

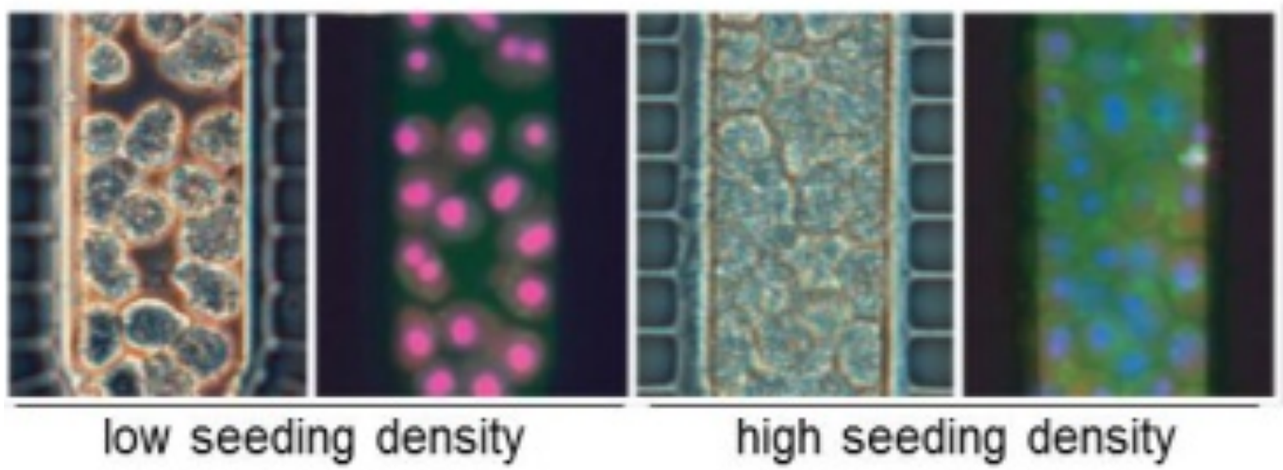
Control System

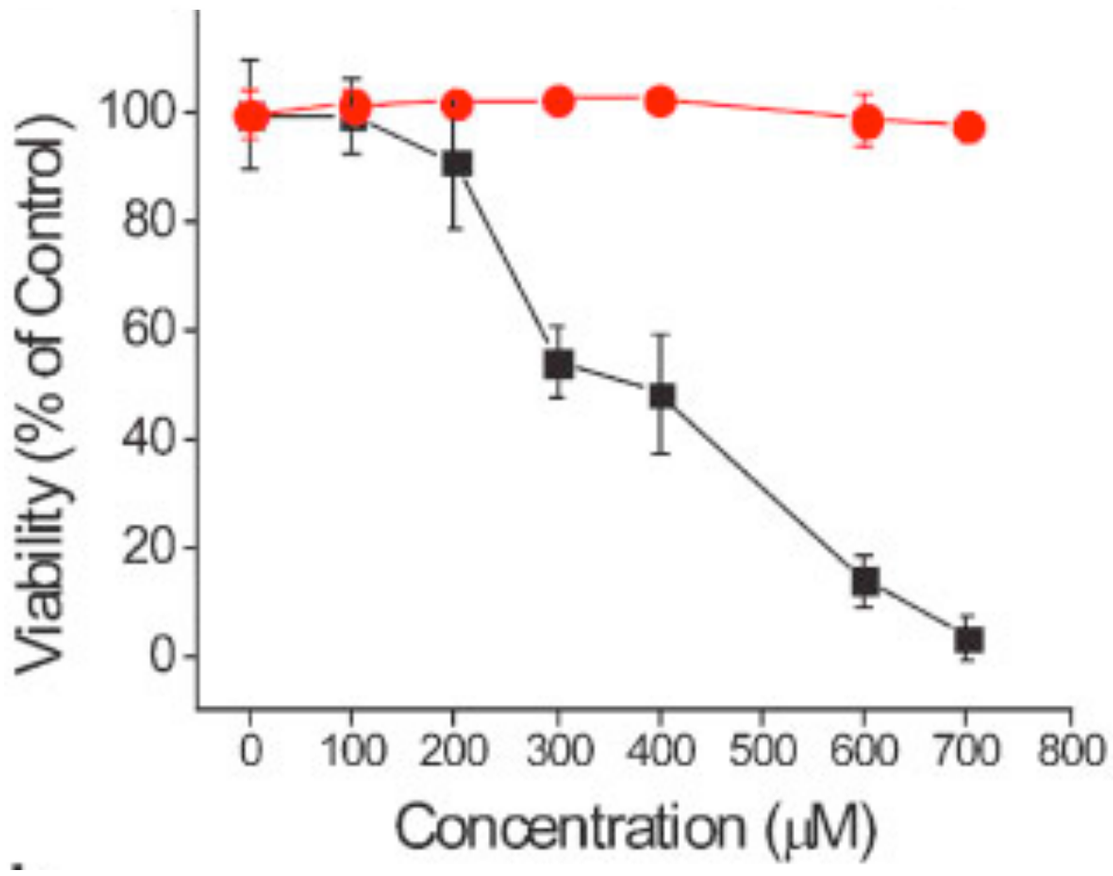
- **Air pressure-driven flow control**
- **CO_2 /gas delivery directly to microfluidic chip**
- **Transparent manifold window for imaging, e.g. DIC**
- **Vacuum sealed micro-incubator**
- **Software schedules media flow**

CELLASIC

Microfluidic Liver Tissue Model Array

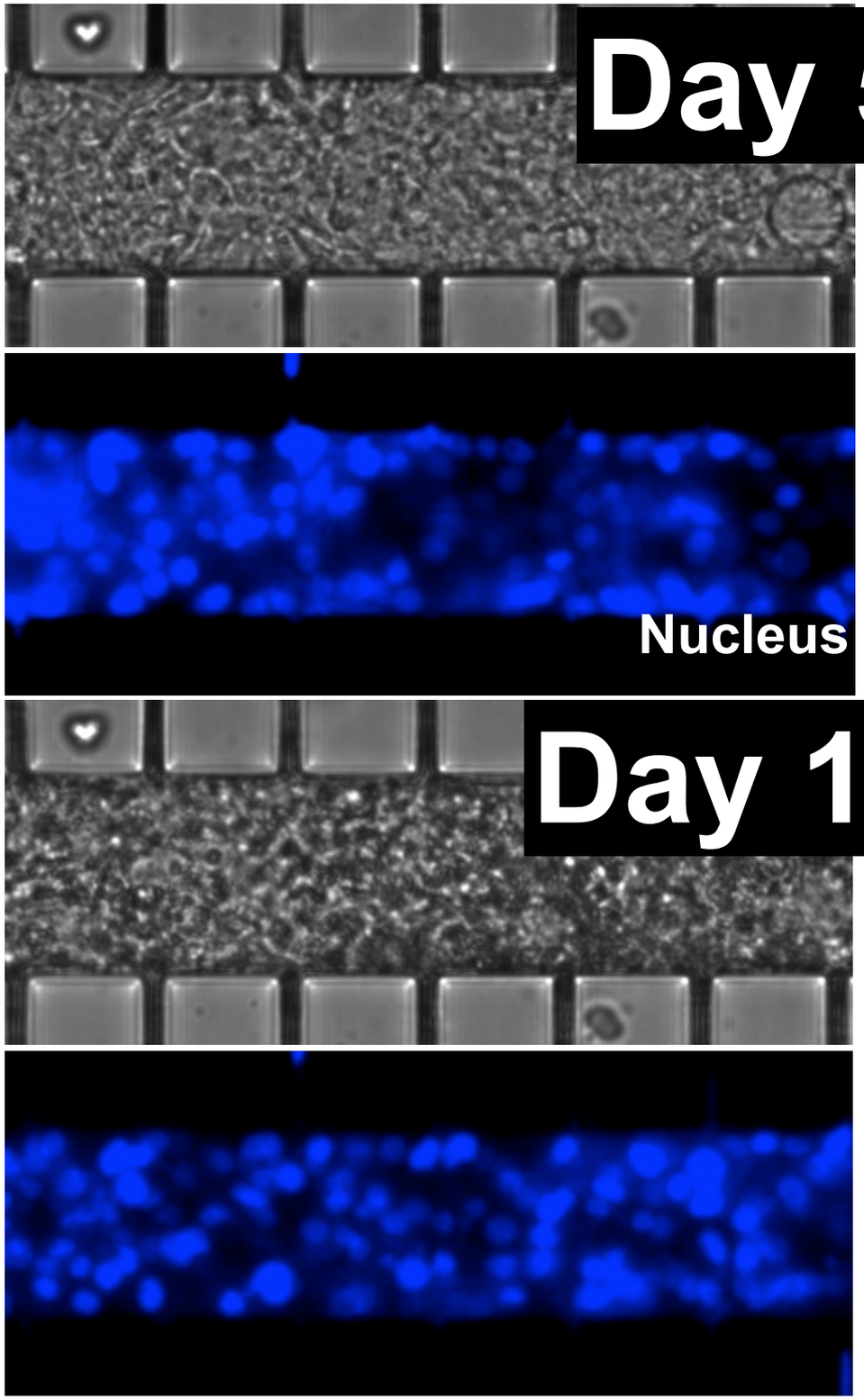


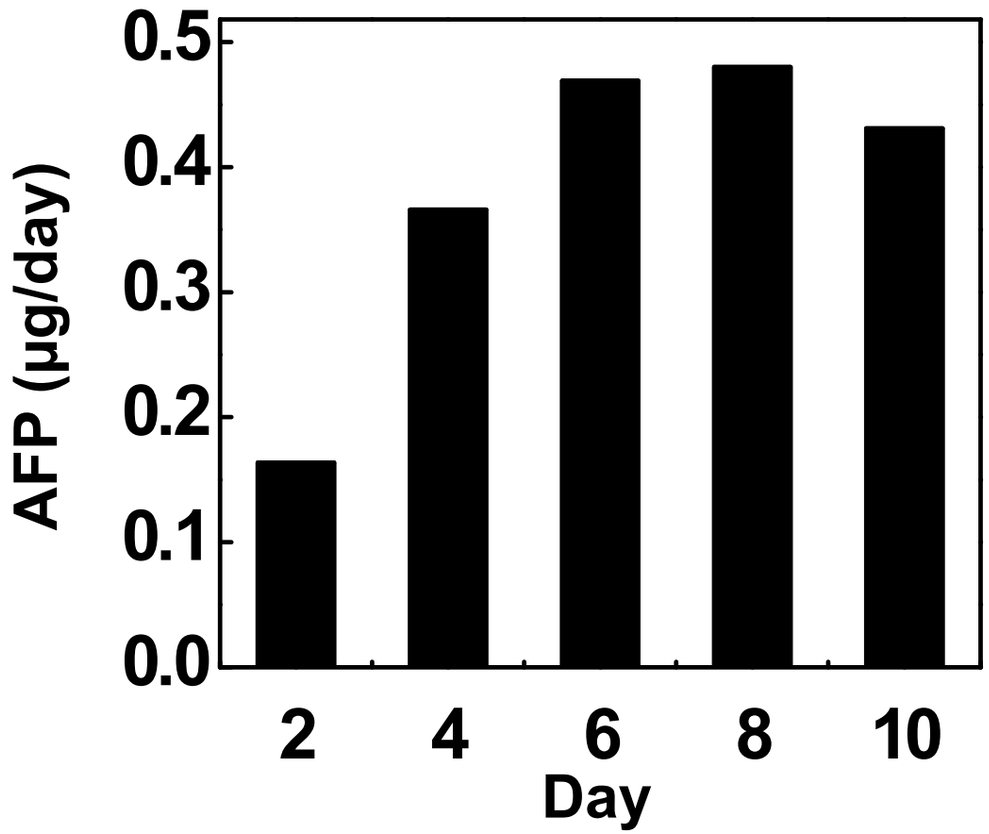




diclofenac was used to test metabolism mediated hepatotoxicity

hHPs on Chip

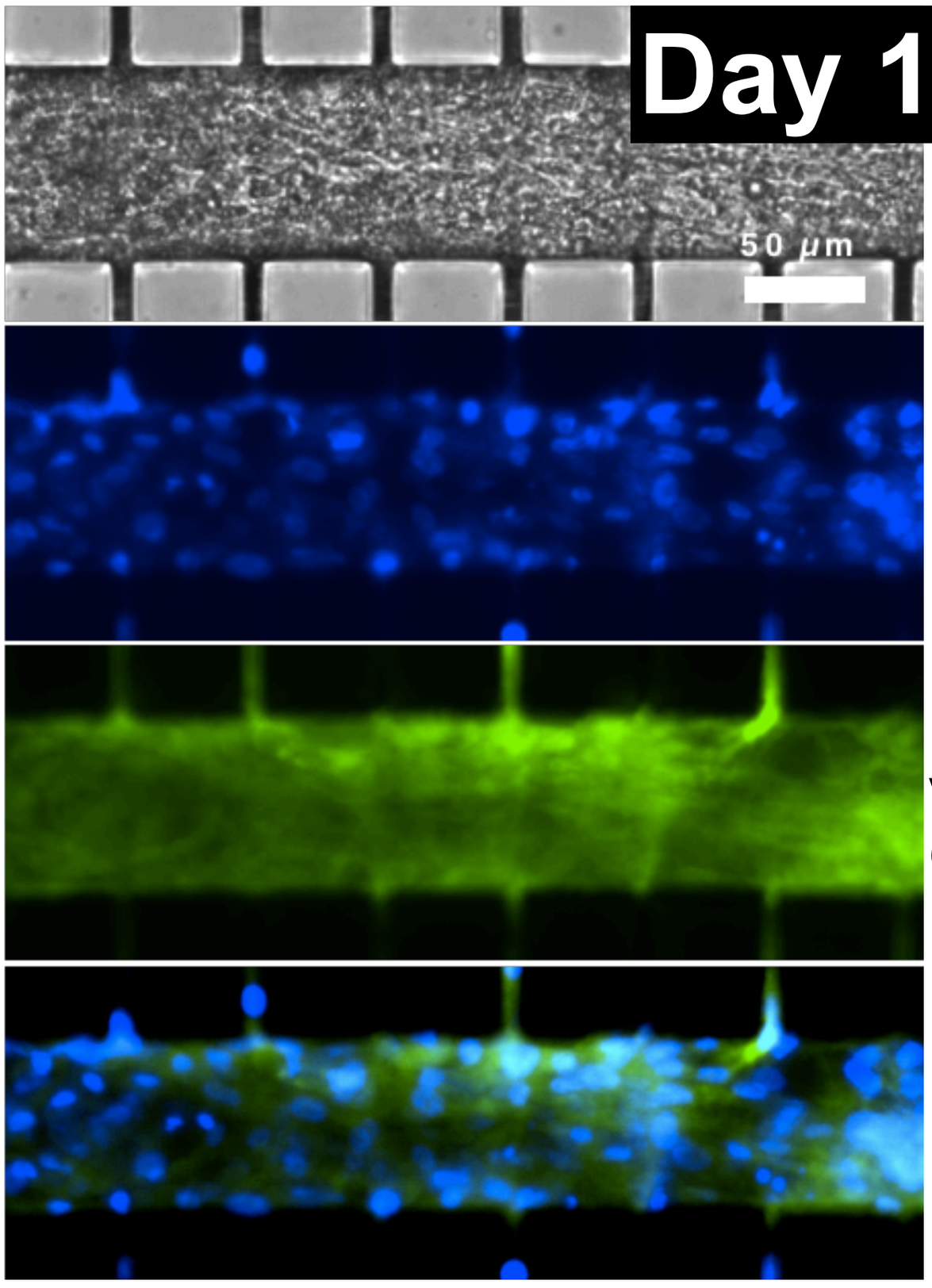




** α-fetoprotein (AFP) secretion per unit (~30,000 cells)*

** Albumin secretion is not significant (less than 10 ng/day)*

hHPs on Chip



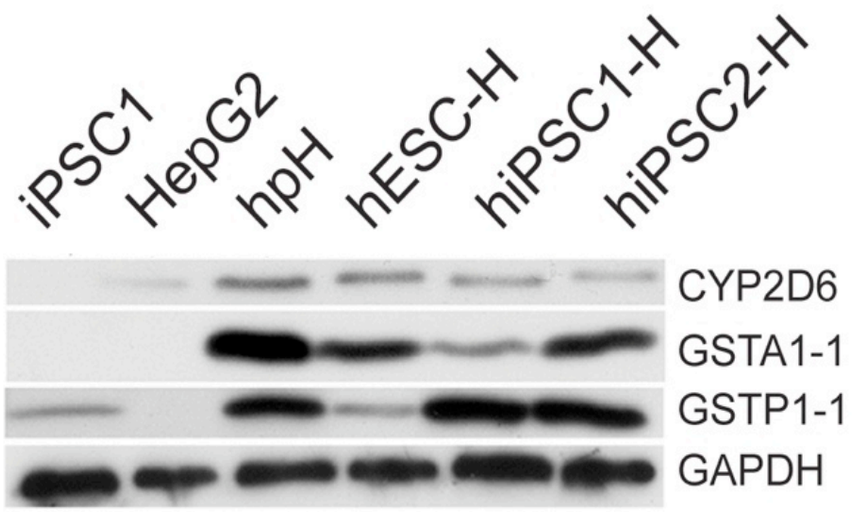
Hepatocytes Derived from Patient-Specific iPSCs

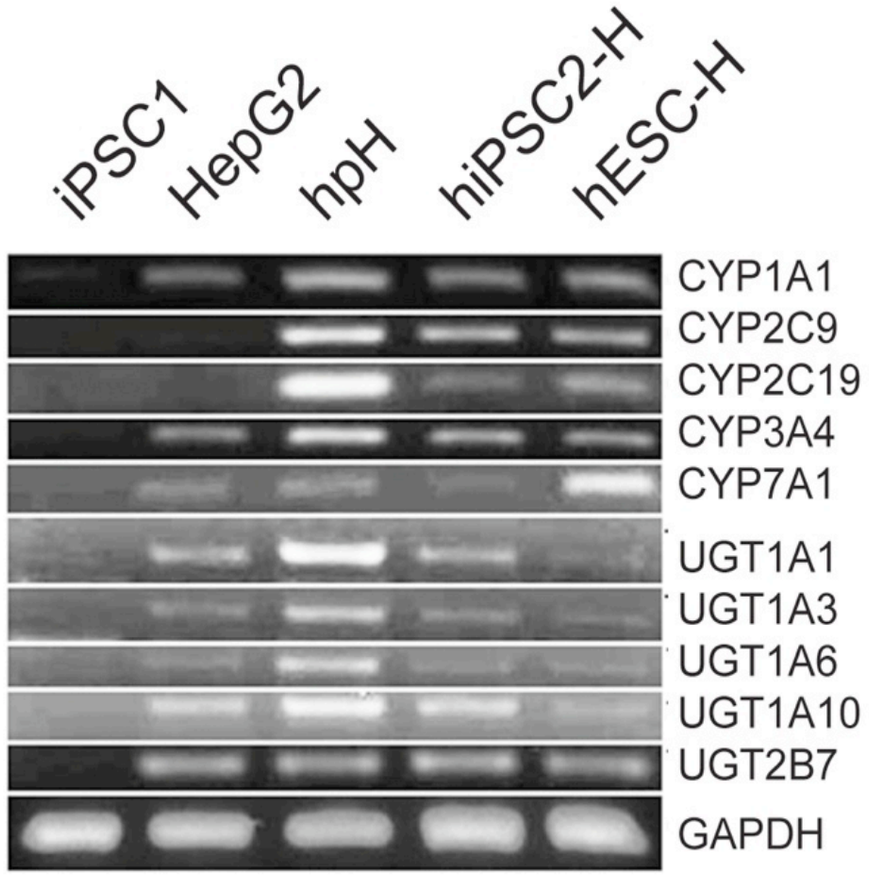
Expression of phase I, II and III drug metabolism enzymes in hiPSC-HPs

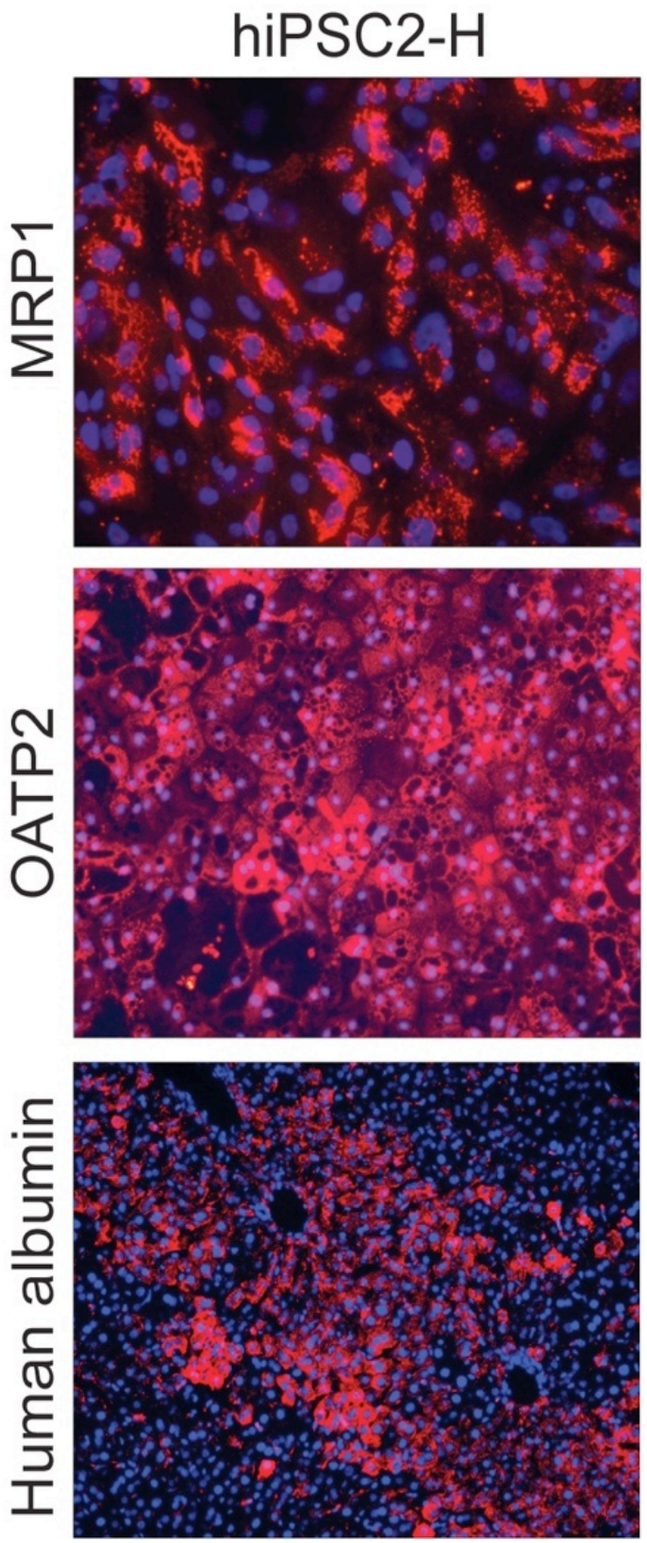
hiPSC-HP line 2 expresses phase I and II enzymes at similar or higher levels than hESC-HPs

Levels of most enzymes are of similar magnitude as those found in hpHPs

Holger Willenbring
UCSF- Stem Cell Center
UCSF- Liver Center







Milestones - Year 1

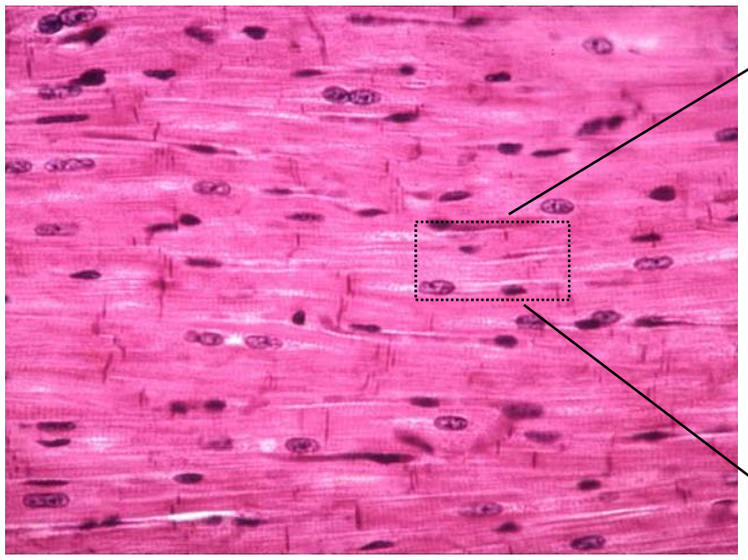
Milestone 2. To organize the structure of healthy and LQTS-hiPSC-CMs into a 3D *in vitro* model of the human myocardium. To assess the functional behavior of the normal and "diseased tissue" models by examining their electrical activity and function.

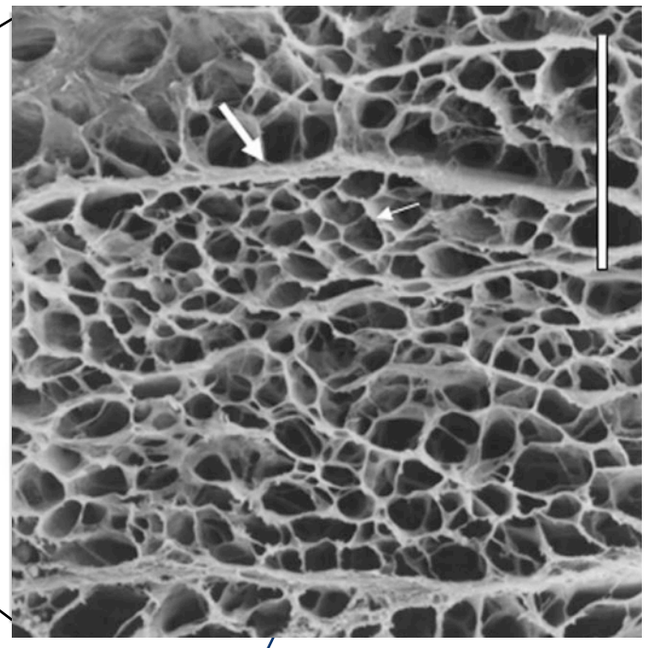
Goals and timeline: Determine a set of device parameters that organize the alignment of hiPSC-CMs into a beating microtissue. 14 months.

Criteria for Success: Healthy hiPSC-CM derived cardiac tissue will have physiologically relevant mean field potential duration (~400 ms) and beat rates (60 beats/min). LQTS hiPSC-CM-derived cardiac tissue will have physiologically relevant mean field potential duration (~600 ms) and beat rates (60 beats/min). Healthy and LQTS hiPSC-CM-derived cardiac tissue in our microsystem will be viable and amenable to continuous monitoring (e.g., MEA) and sampling for over 4 weeks.

Device parameters will affect cell-cell contacts, electrical activity, and contraction within the 3D tissue formed

CM cells in the heart

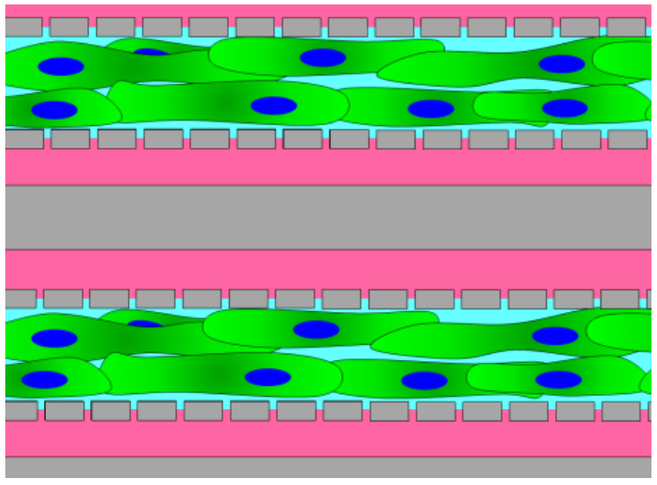




Perimysial collagen fibers aligned with CMs

Kanzaki, Y. et al., Circulation. 2010;122:1973-1974.

hiPS-CM cells on-a-chip



Disease-Specific Human Cardiac Tissue Model

Inlet for hiPS-CMs

Inlet for medium

Integrated multiple electrode arrays (MEAs) for effective stimulation, recording, and regulation of cardiac beat frequency

Outlet

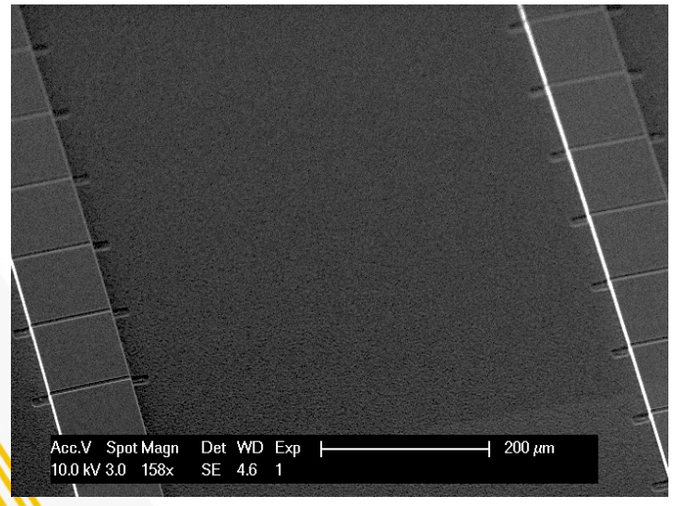
Convective transport

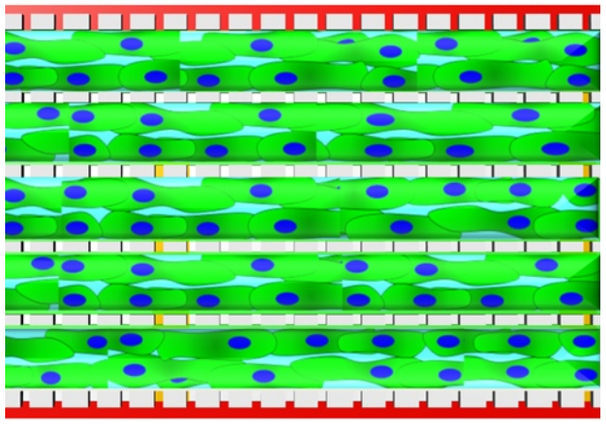
$$\frac{\partial C}{\partial t} + v \times \nabla C = D_{ij} \nabla^2 C$$

Flow control of medium

Diffusive transport

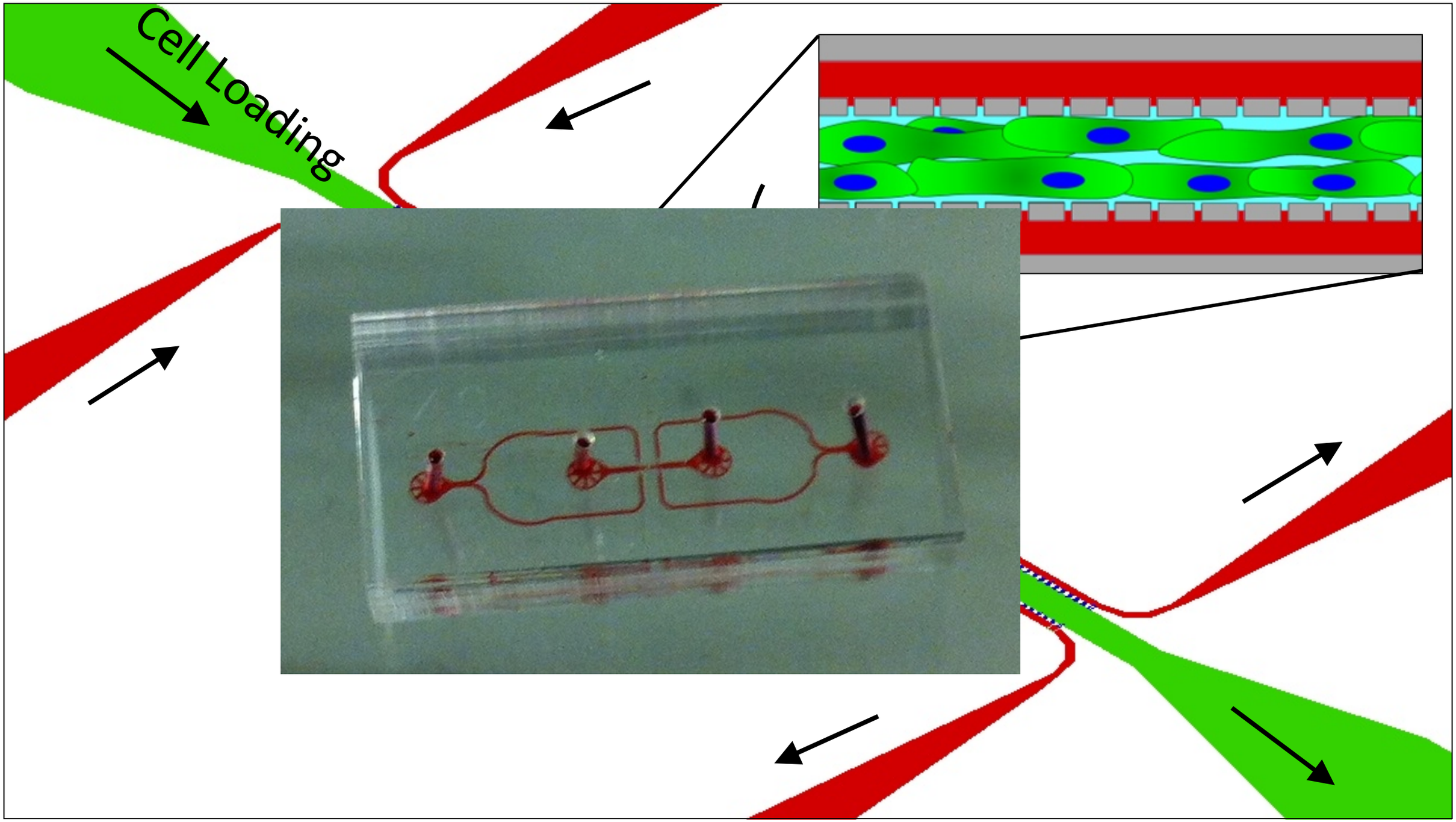
$$-V \frac{dC}{dt} = A_m D_m \frac{2(C_1 - C_0)}{L}$$



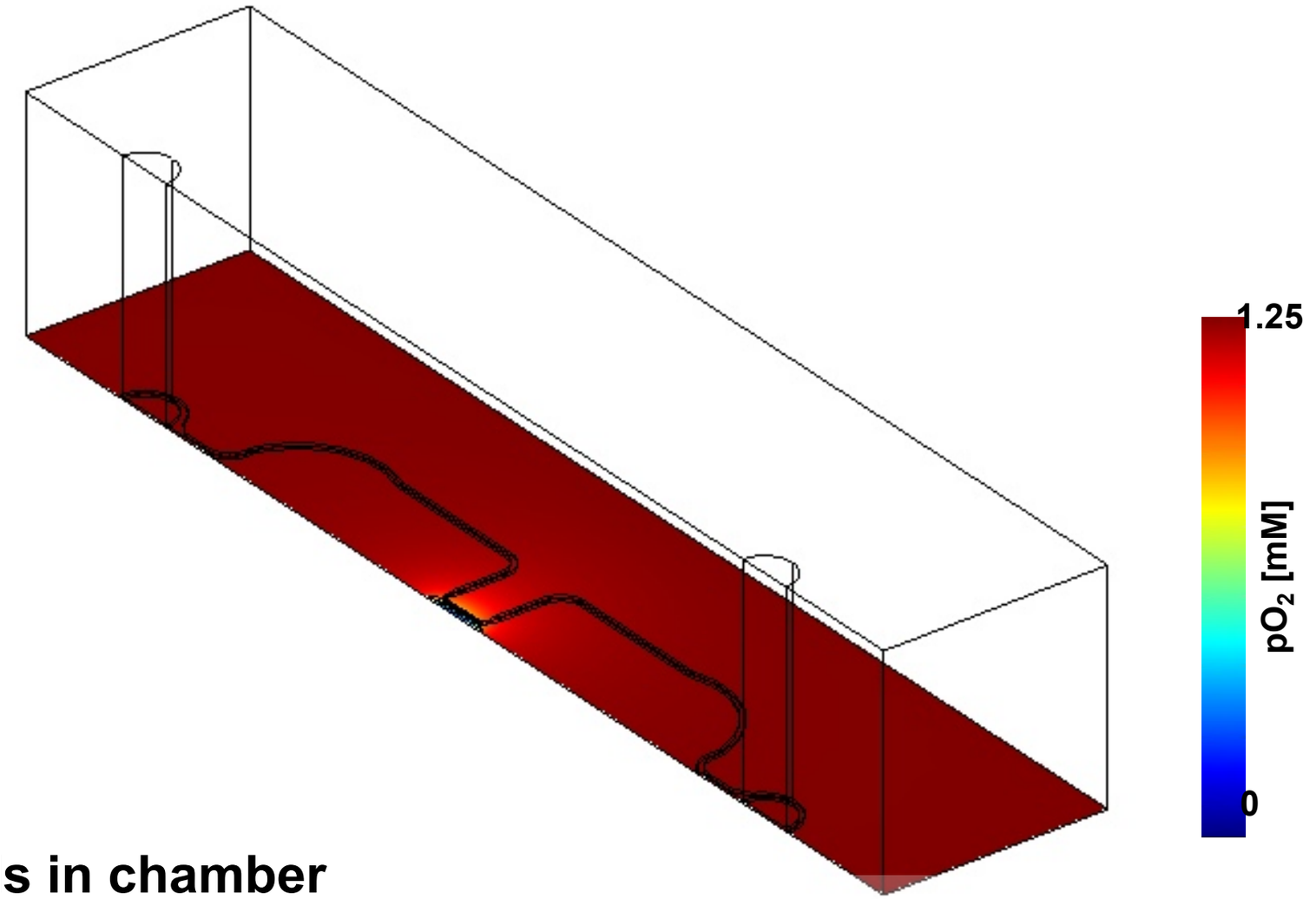


hiPS-CM cells on-a-chip

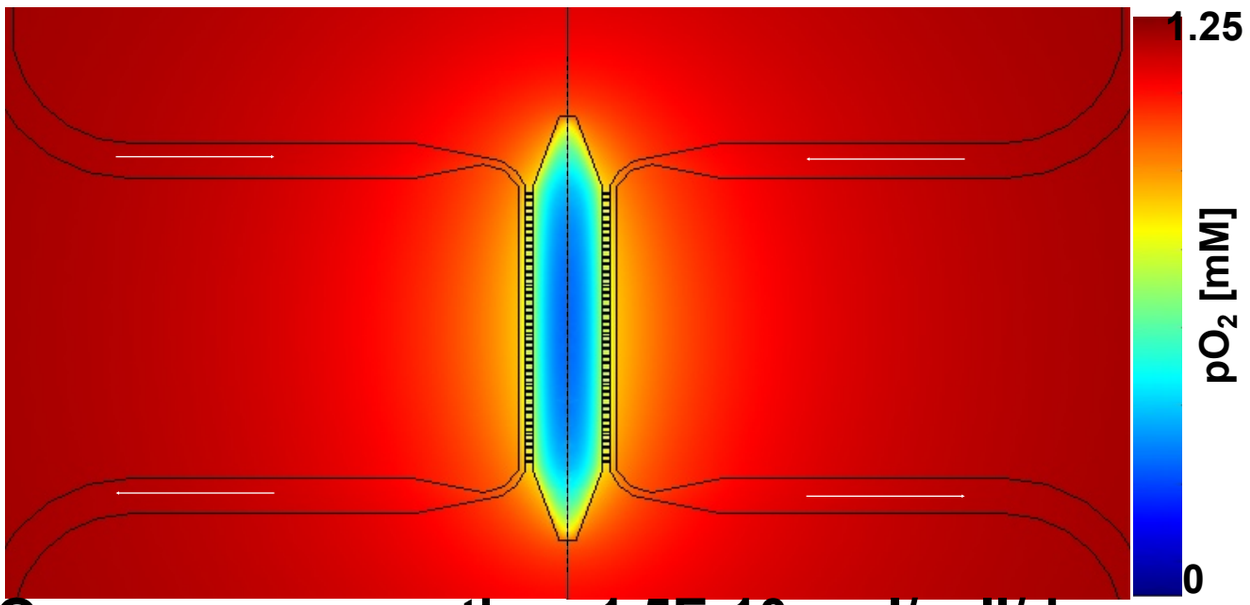
Disease-Specific Human Cardiac Tissue Model



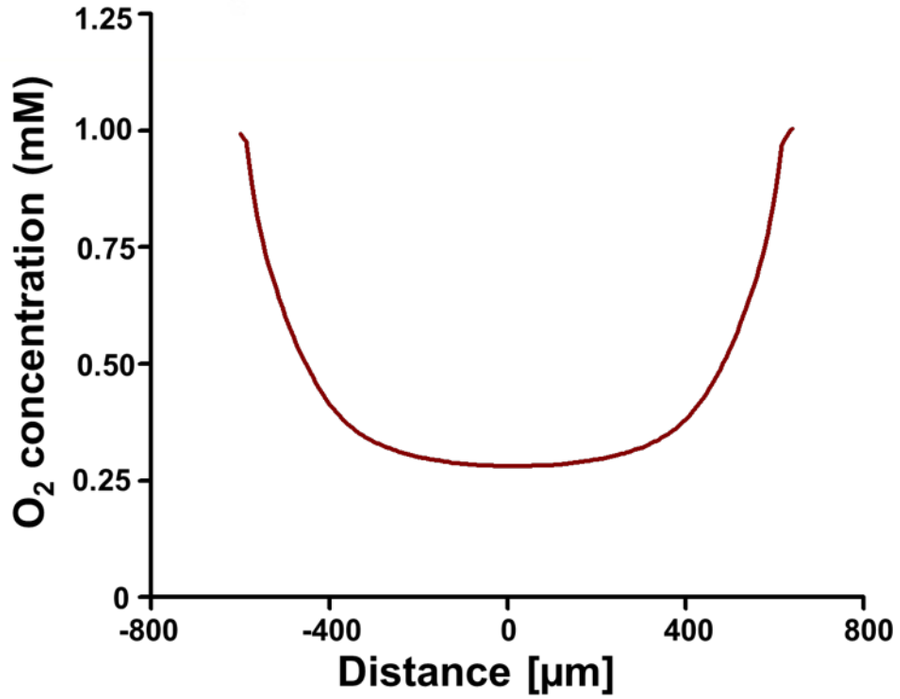
Disease-Specific Human Cardiac Tissue Model



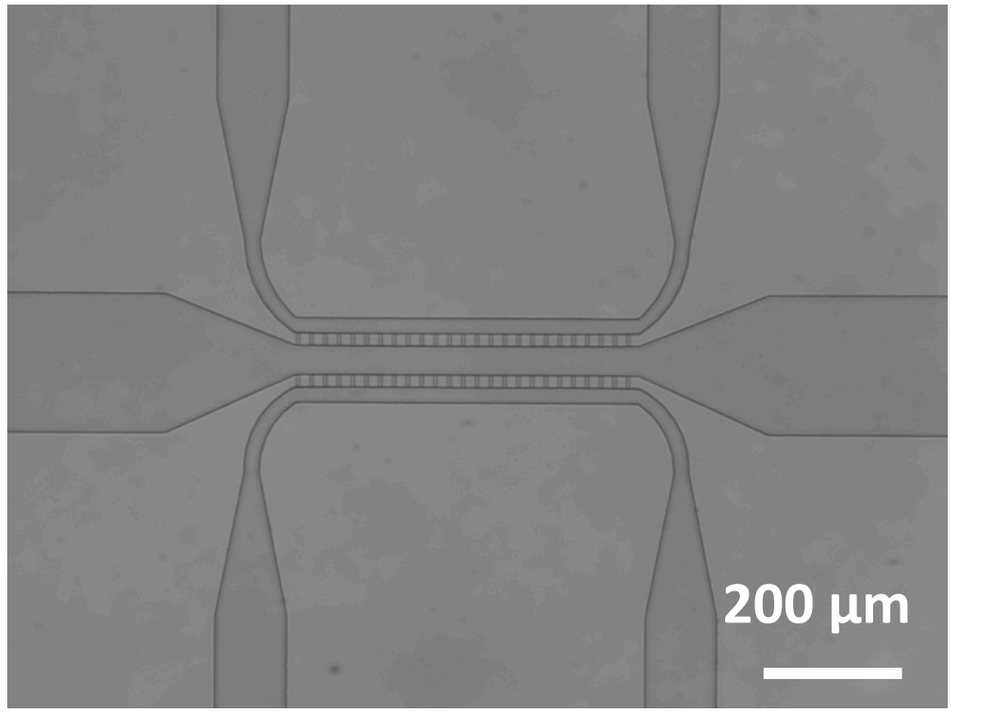
5000 cells in chamber



Oxygen consumption: 1.5E-10 mol/cell/day



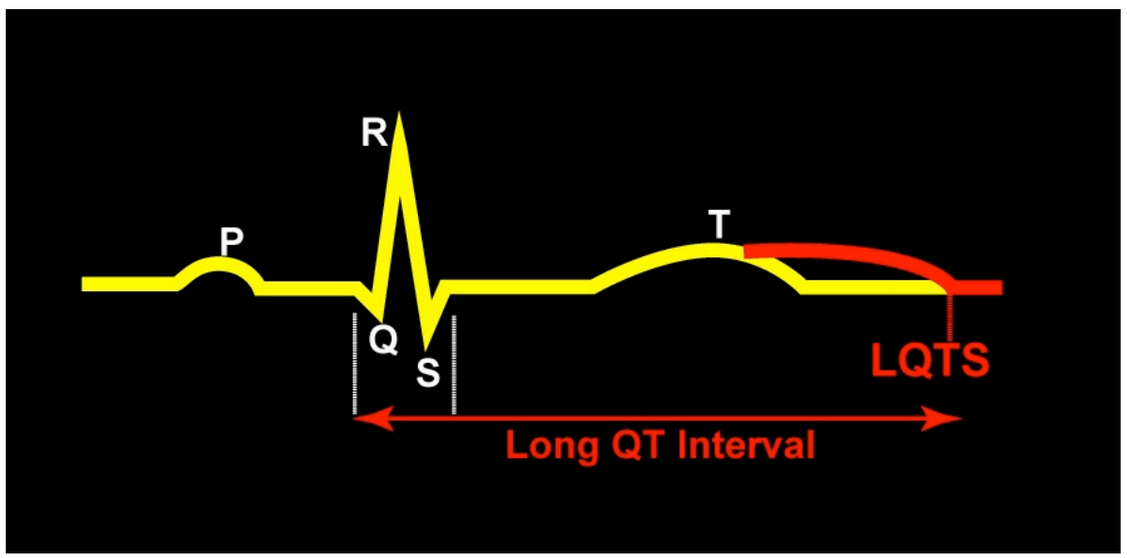
hiPS-CMs on Chip



Long QT Syndrome

Mutations in 10 genes: *KCNQ1* (**LQT1**), *KCNH2* (**LQT2**, HERG), and *SCN5A* (**LQT3**)

ECG



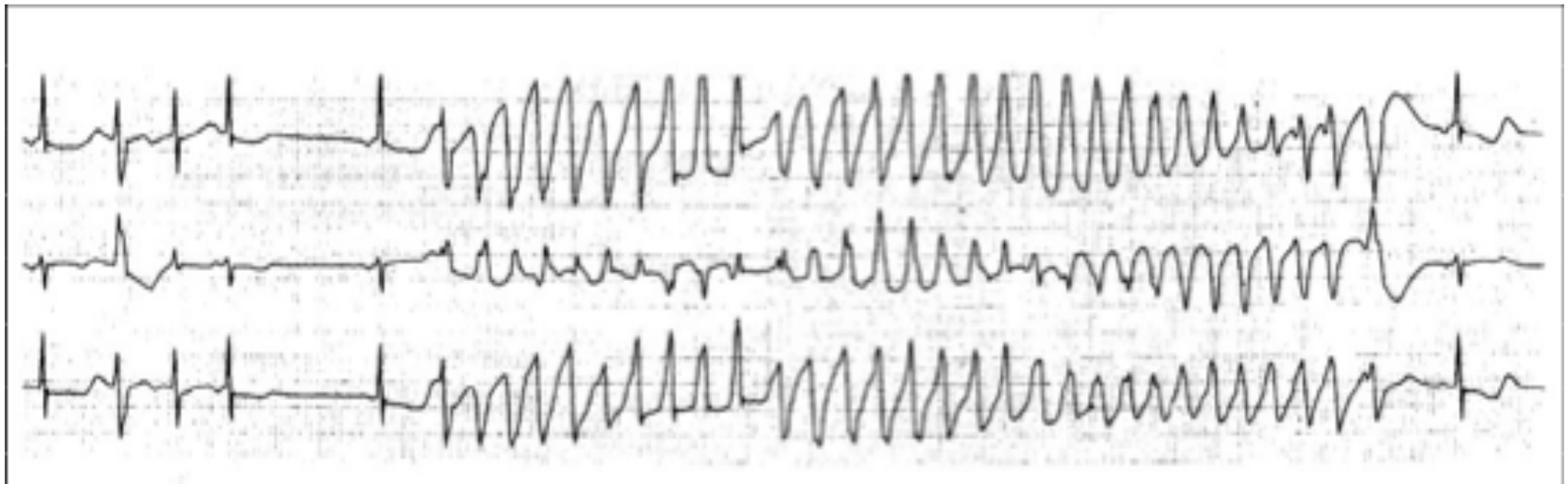
Genetic and drug-induced forms

Genetic prevalence ~ 1 : 2,000

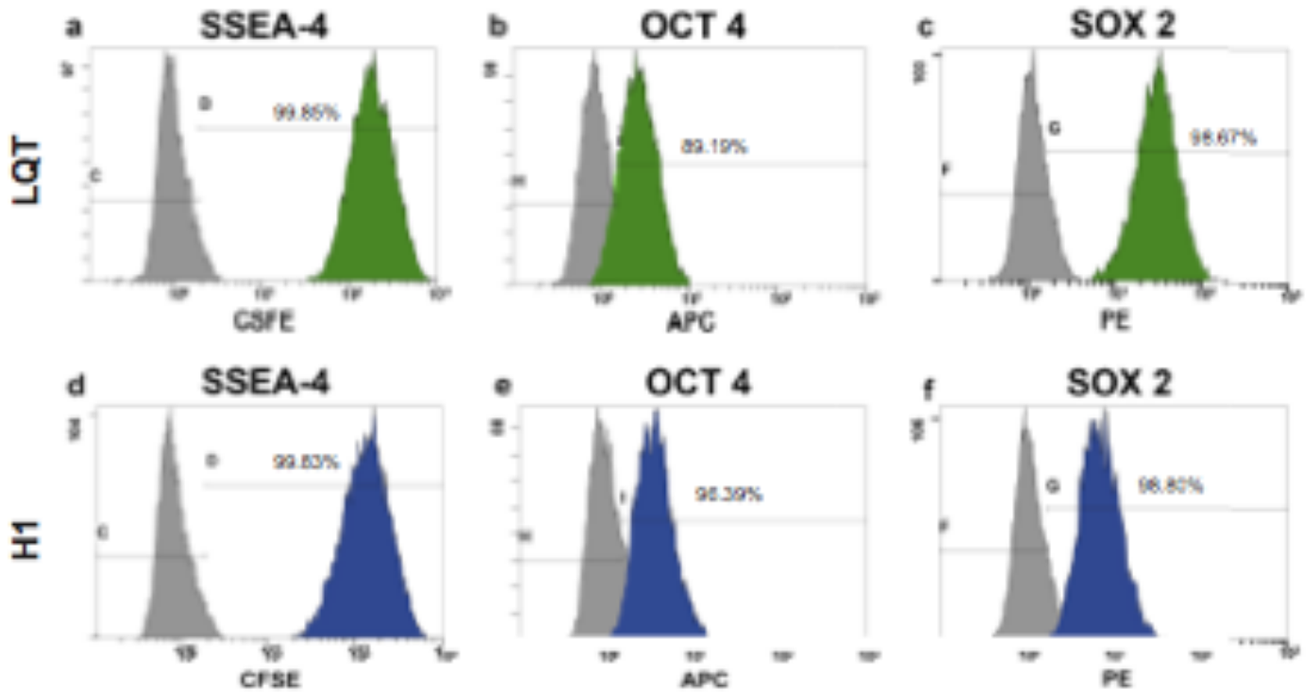
LQT3 patient

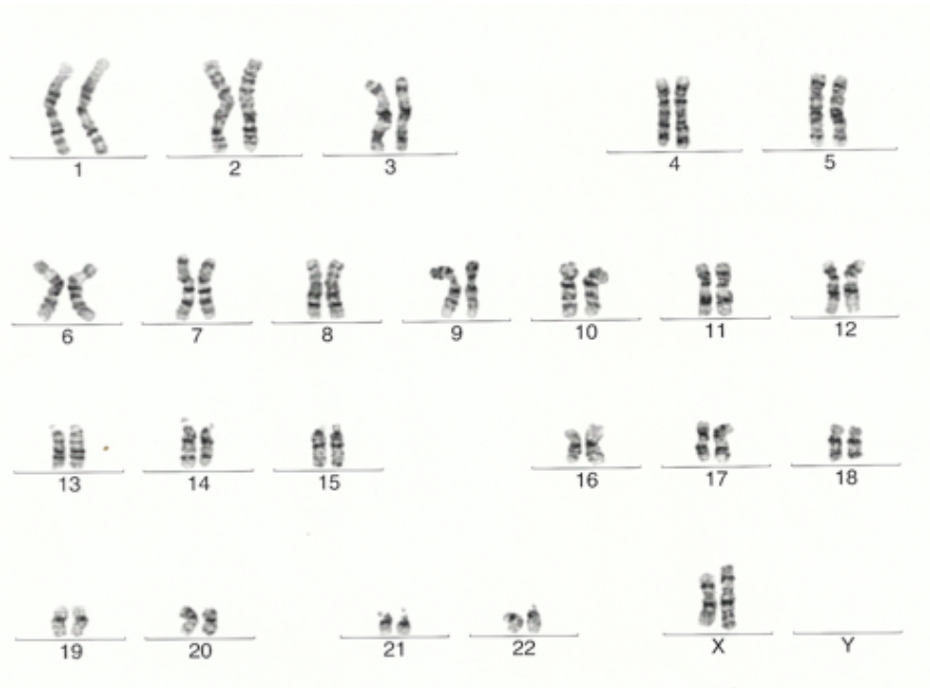
- **Ventricular tachycardia at birth**
- **Na ion channel *SCN5A* N406K mutation**
- **QT interval = 500-523 msec (normal <460)**
- **Many ventricular arrhythmias**
- **Diseased at age 19**

Life-threatening arrhythmias

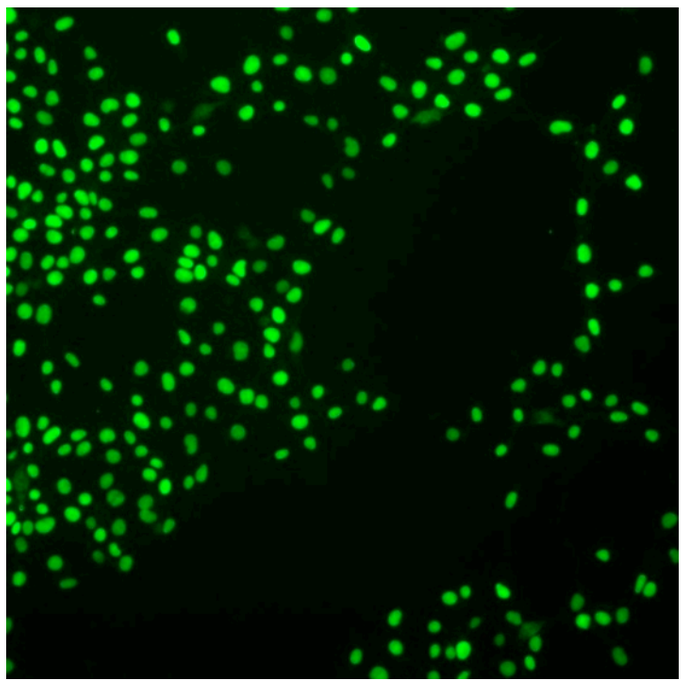


Characterization of LQT hiPSCs

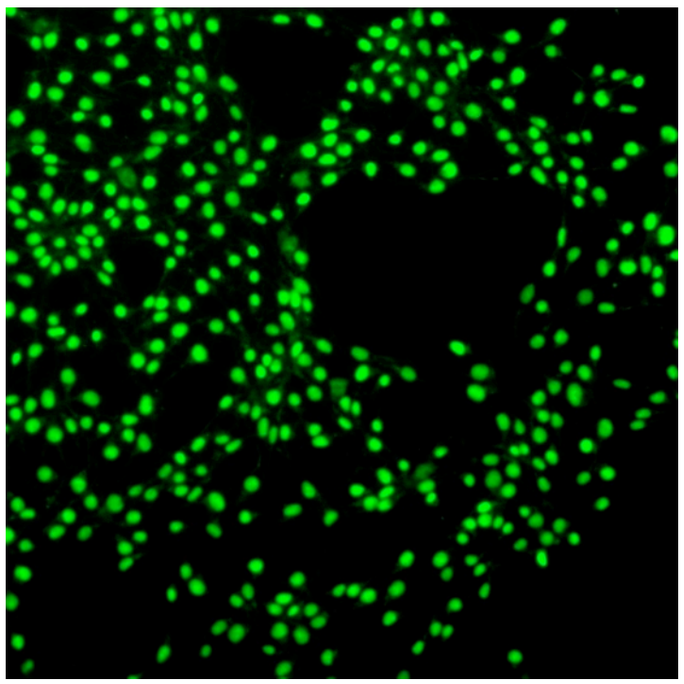




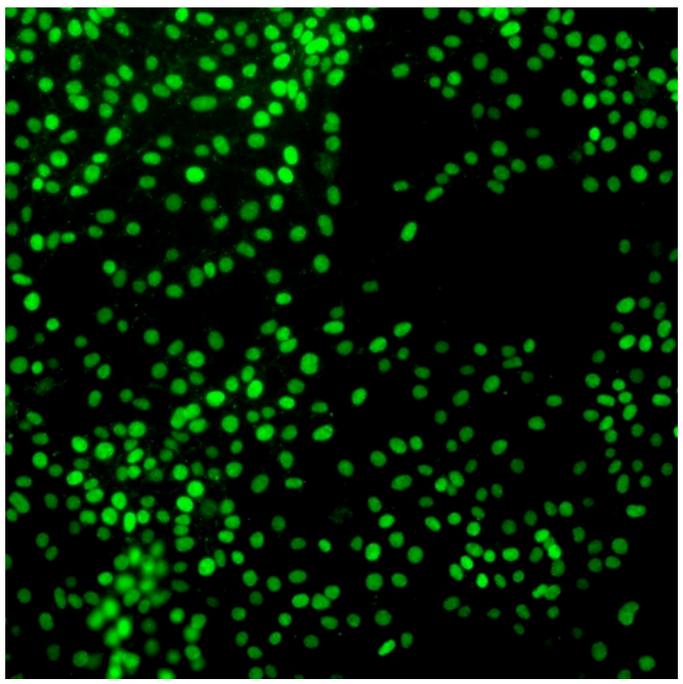
Oct4



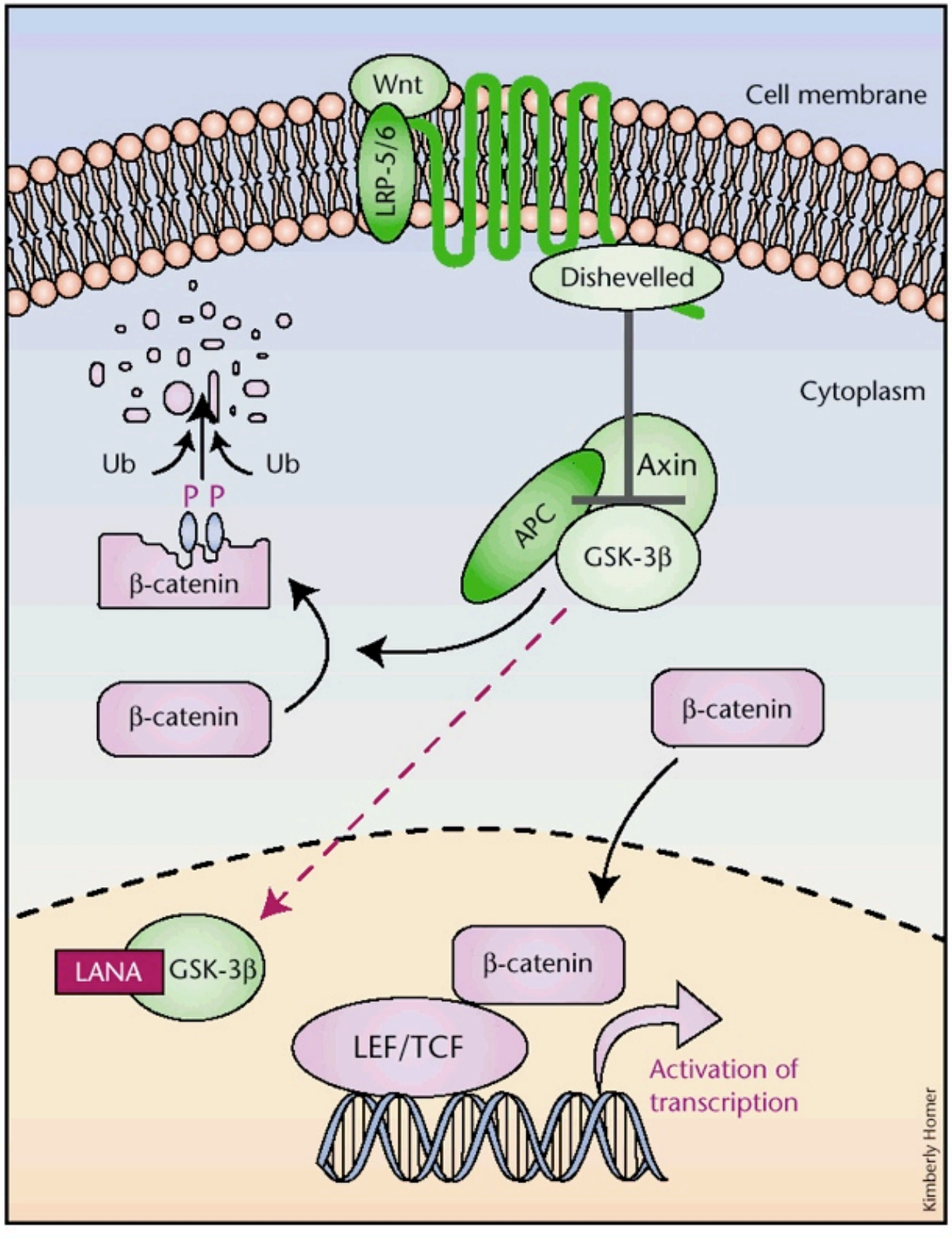
Sox2

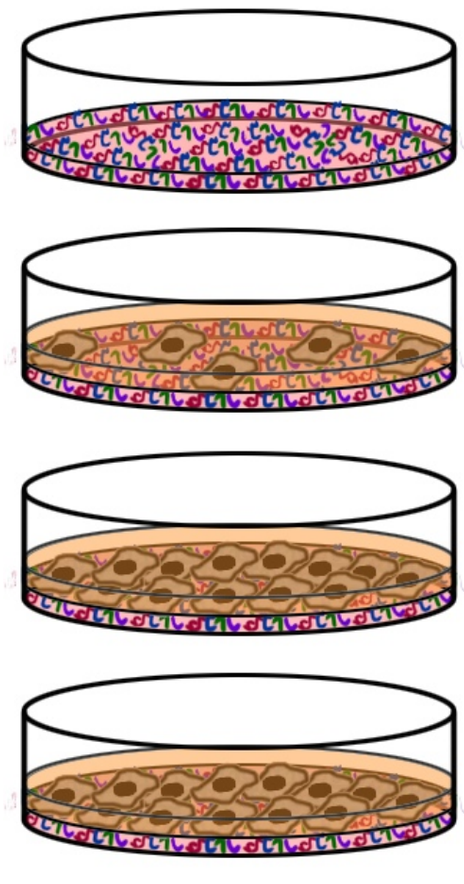


Nanog



Wnt Differentiation Method





Matrigel™ coating

DAY -3: Seed cells in mTeSR™ 1 with ROCK inhibitor

Allow cells to proliferate

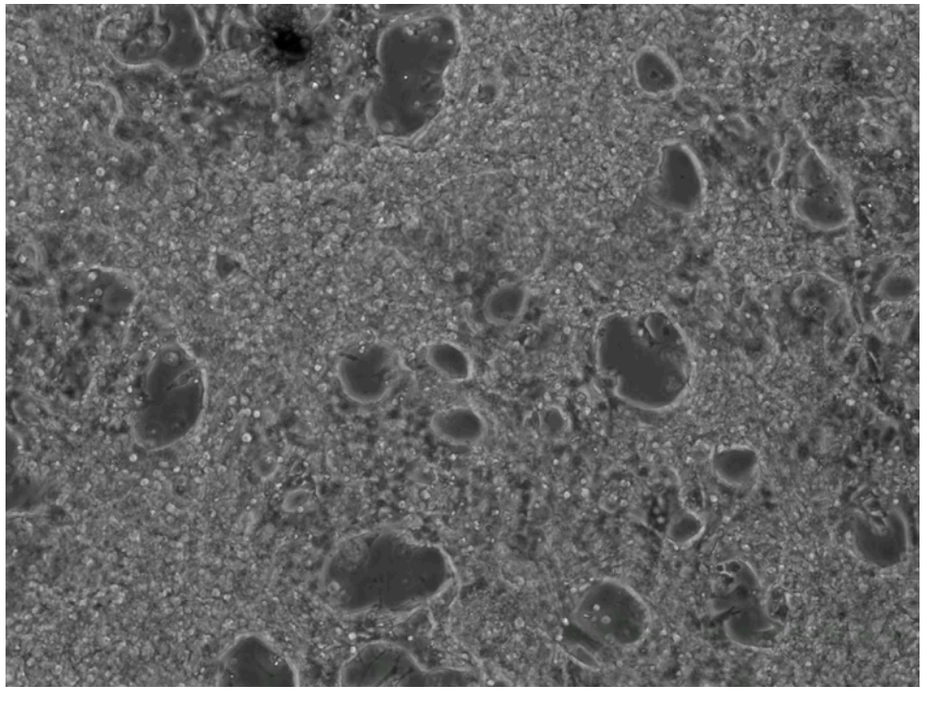
DAY 0: 12 µM CHIR99021 in RPMI/B27-I

DAY 1: RPMI/B27-I

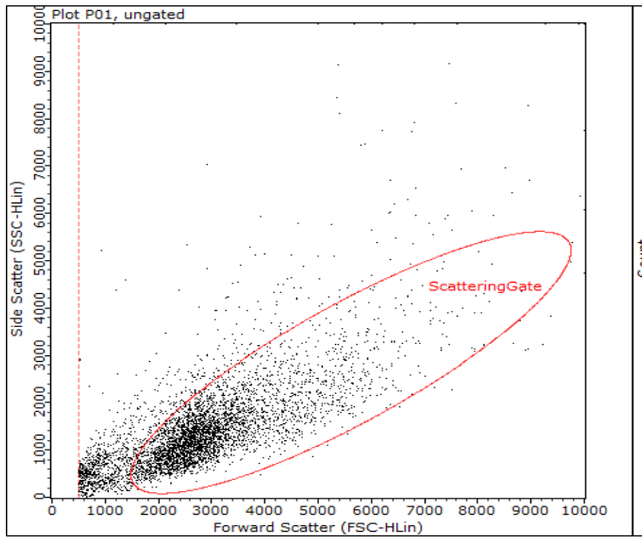
DAY 3: 5 µM IWP-4

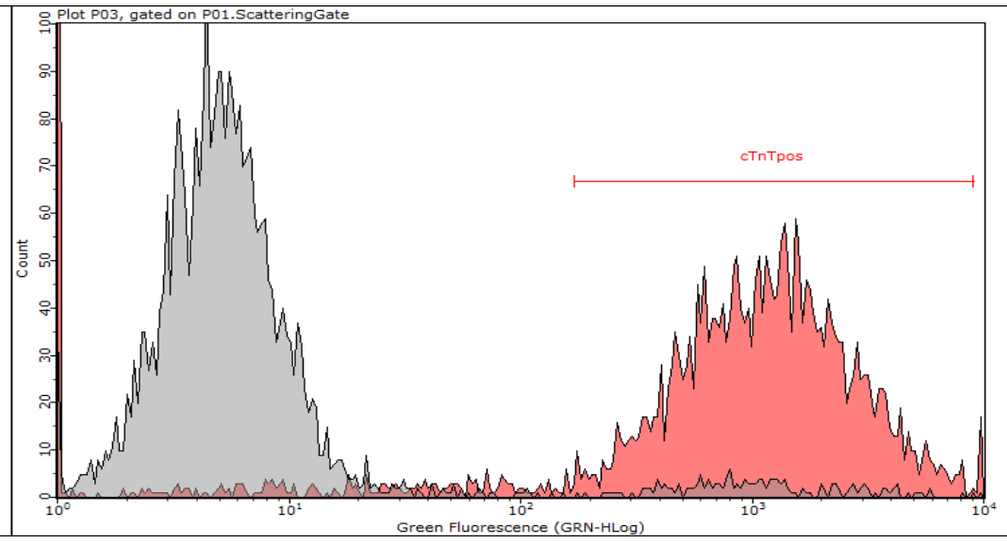
DAY 5: RPMI/B27-I

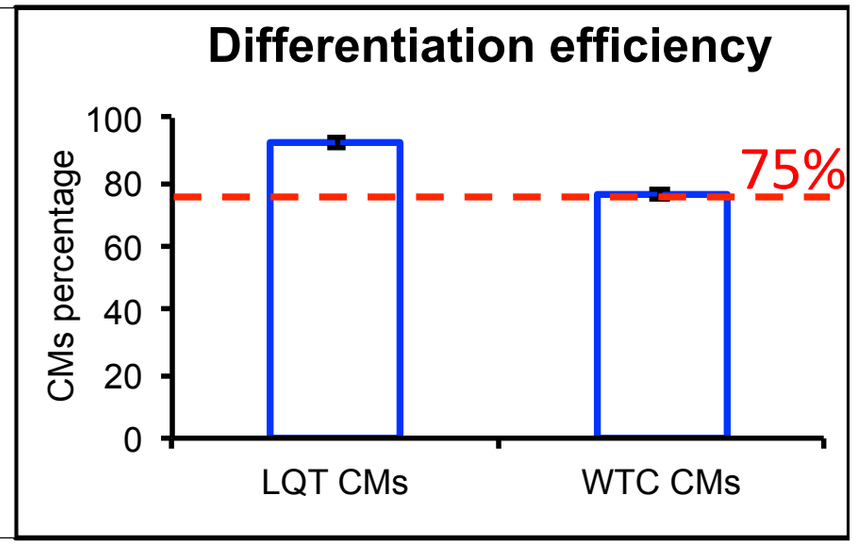
DAY 7 - 30: RPMI/B27 complete supplement

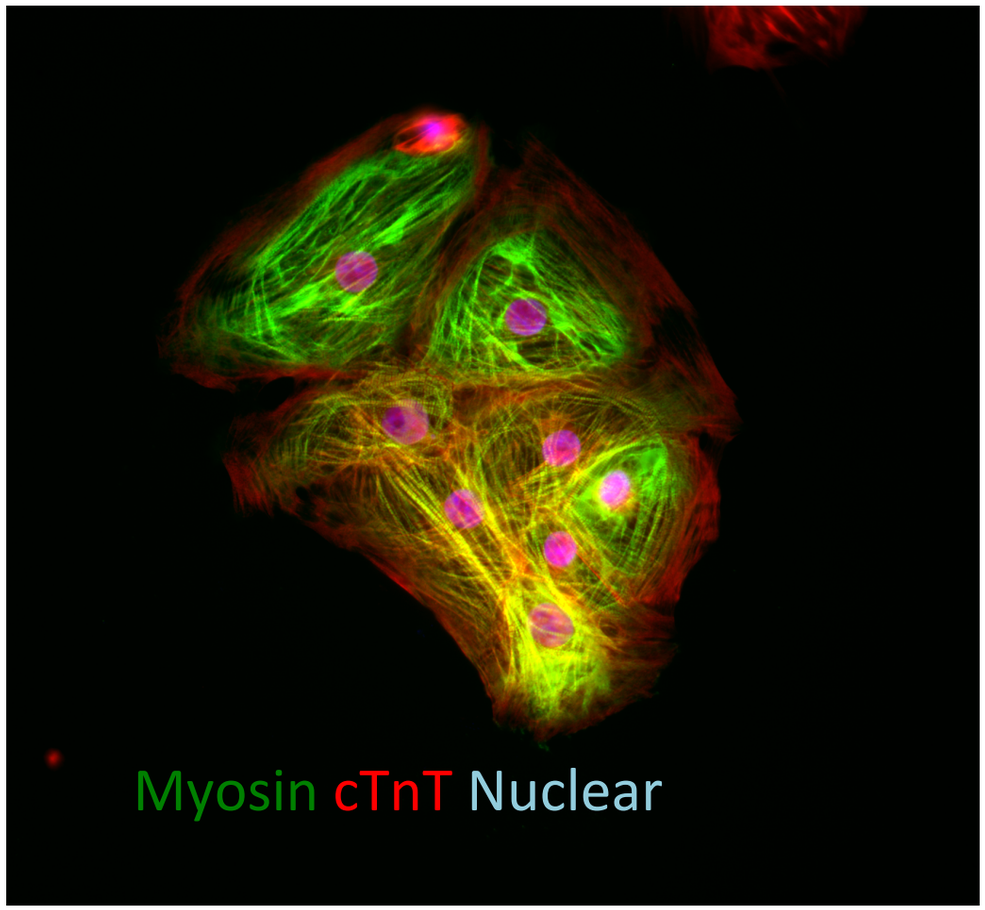


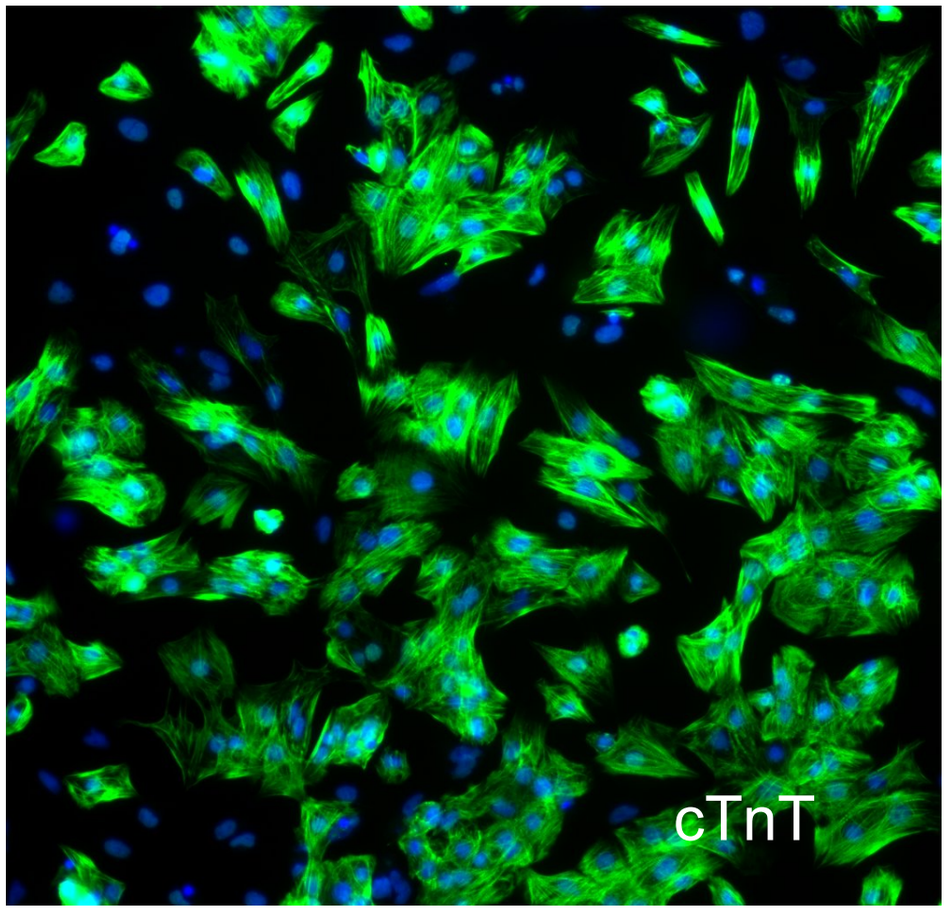
Differentiation Efficiency using WNT Protocol for WT and LQT iPSCs



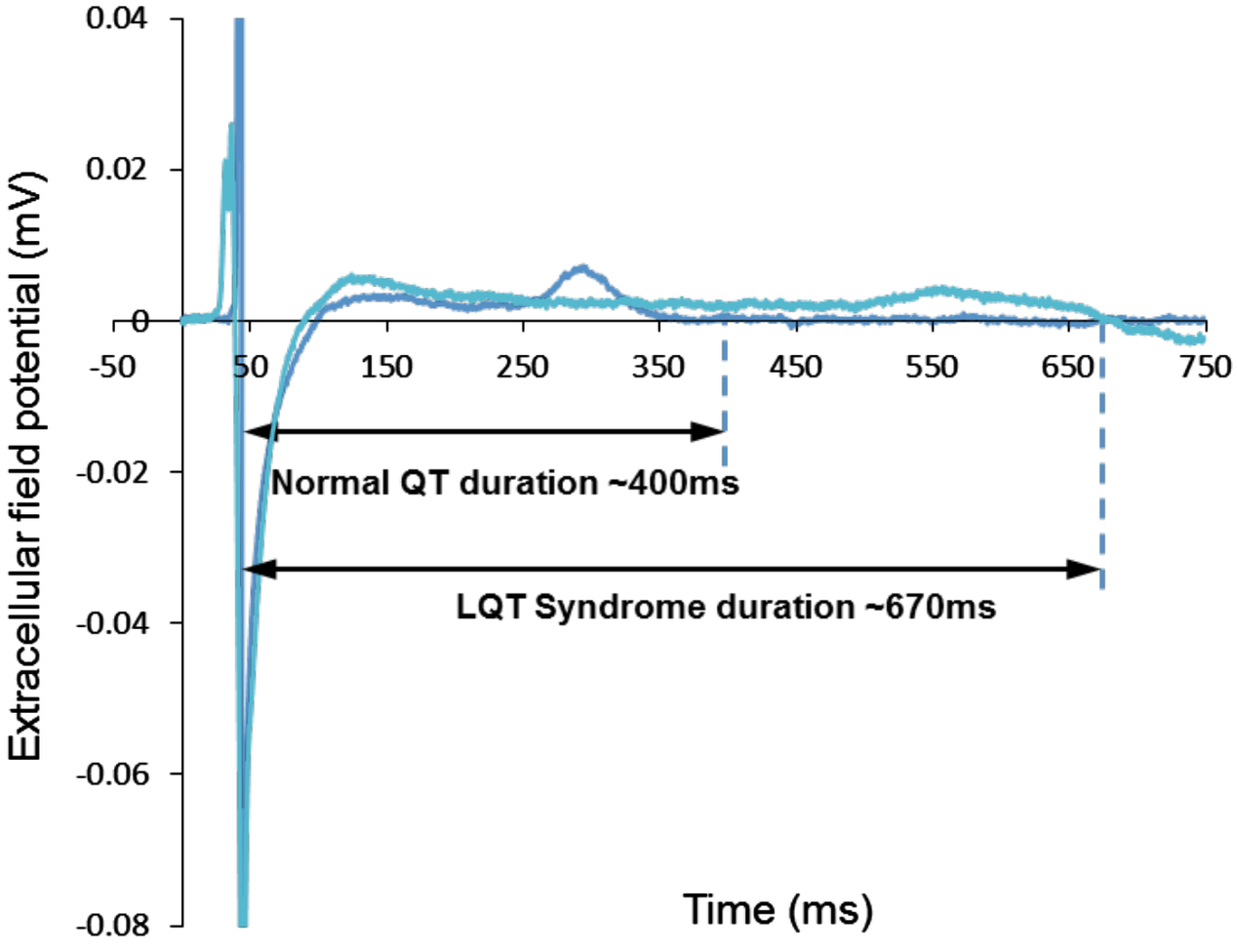


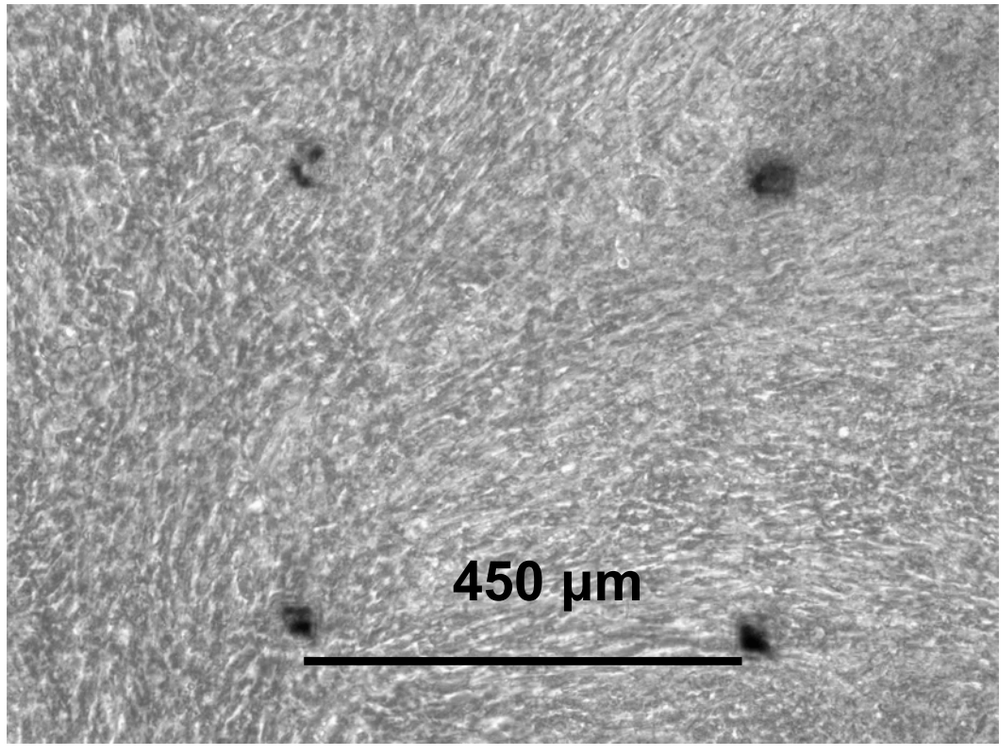


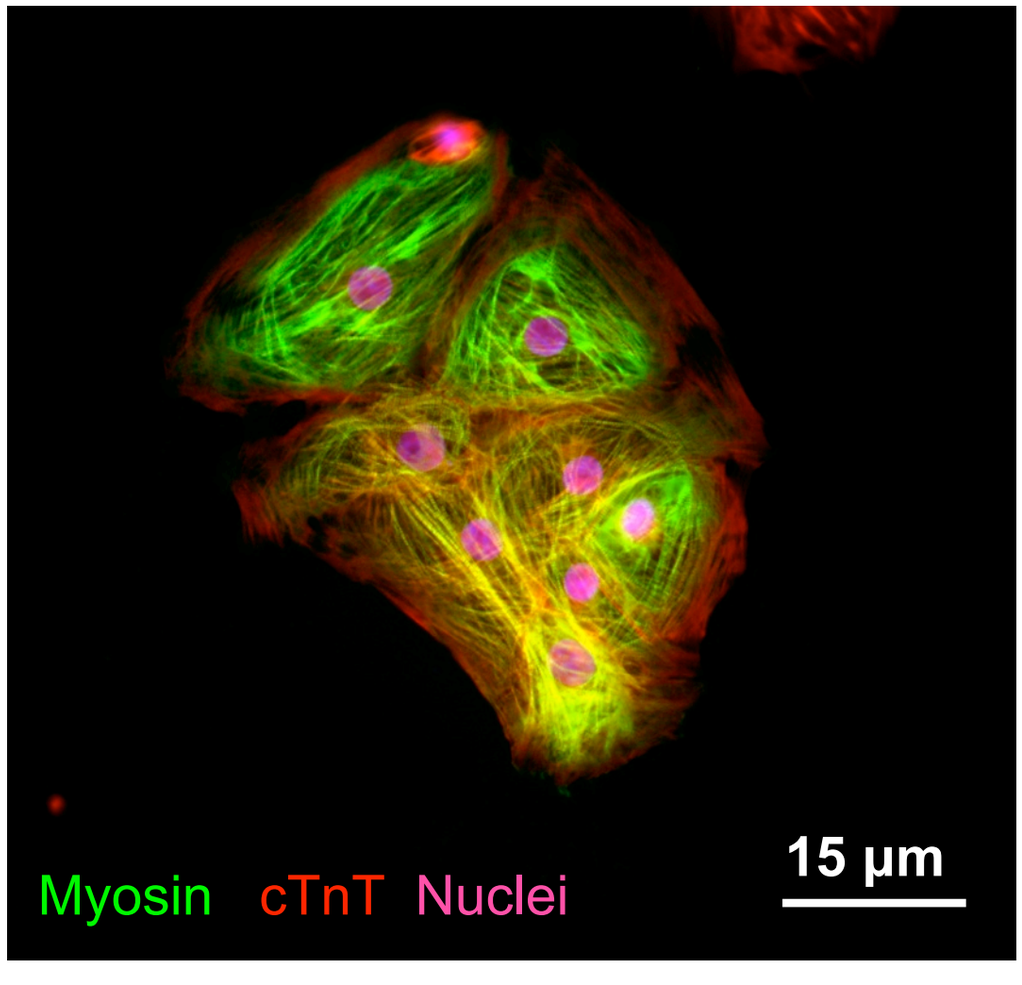




LQT3 hiPSC-CM derived Cardiac Tissue

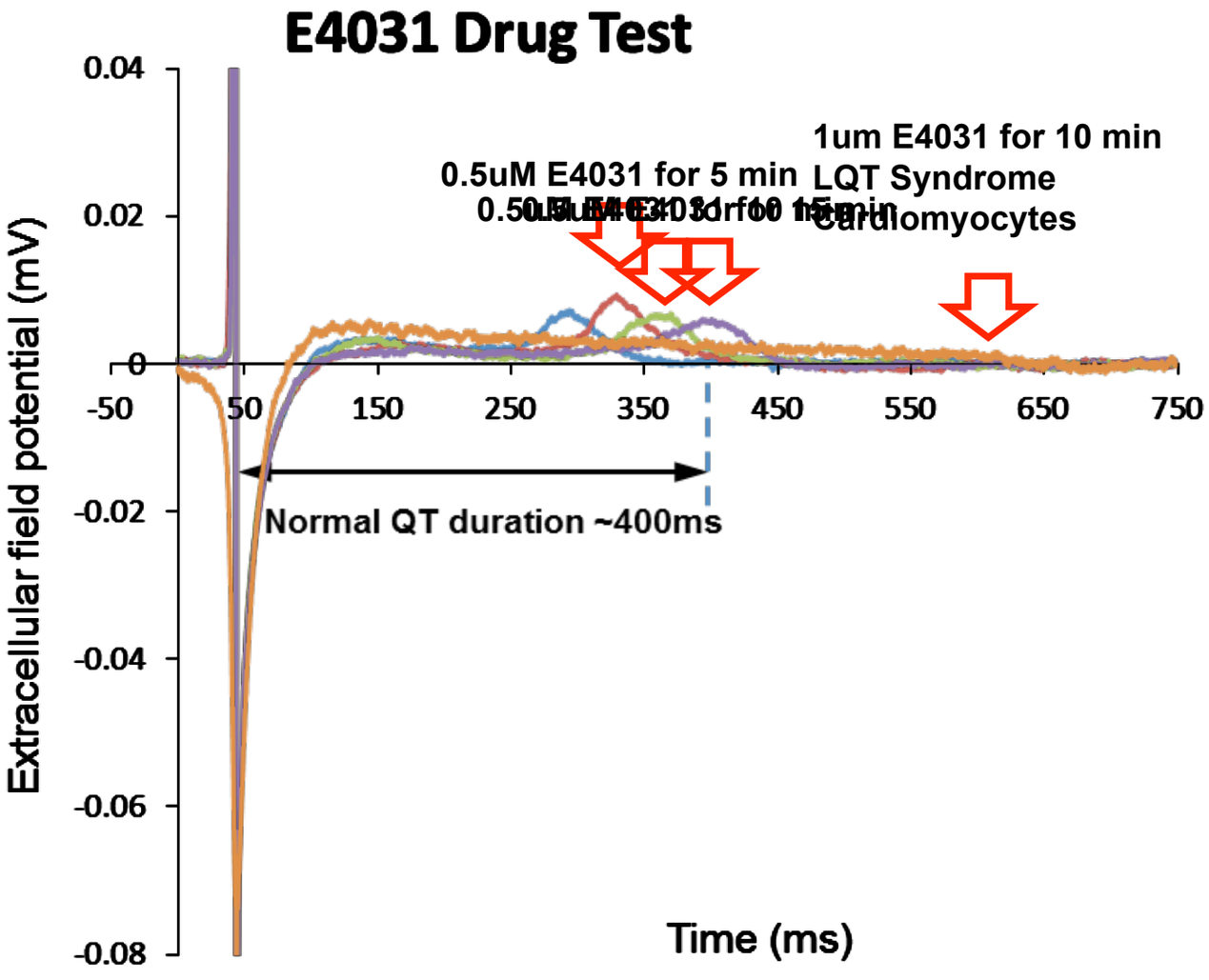


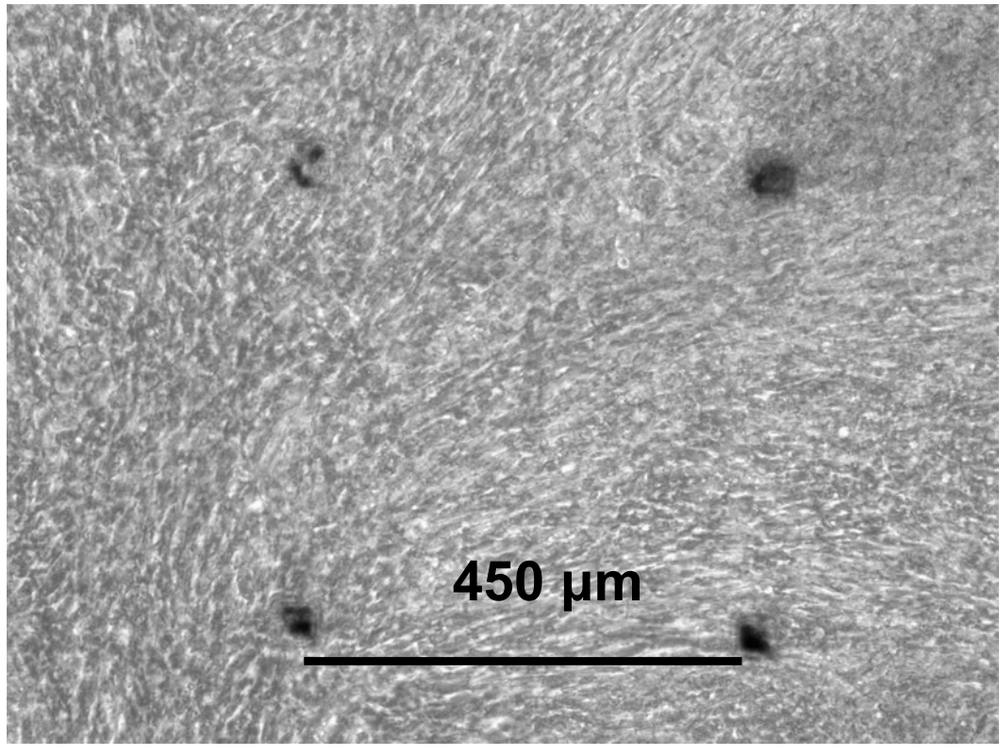


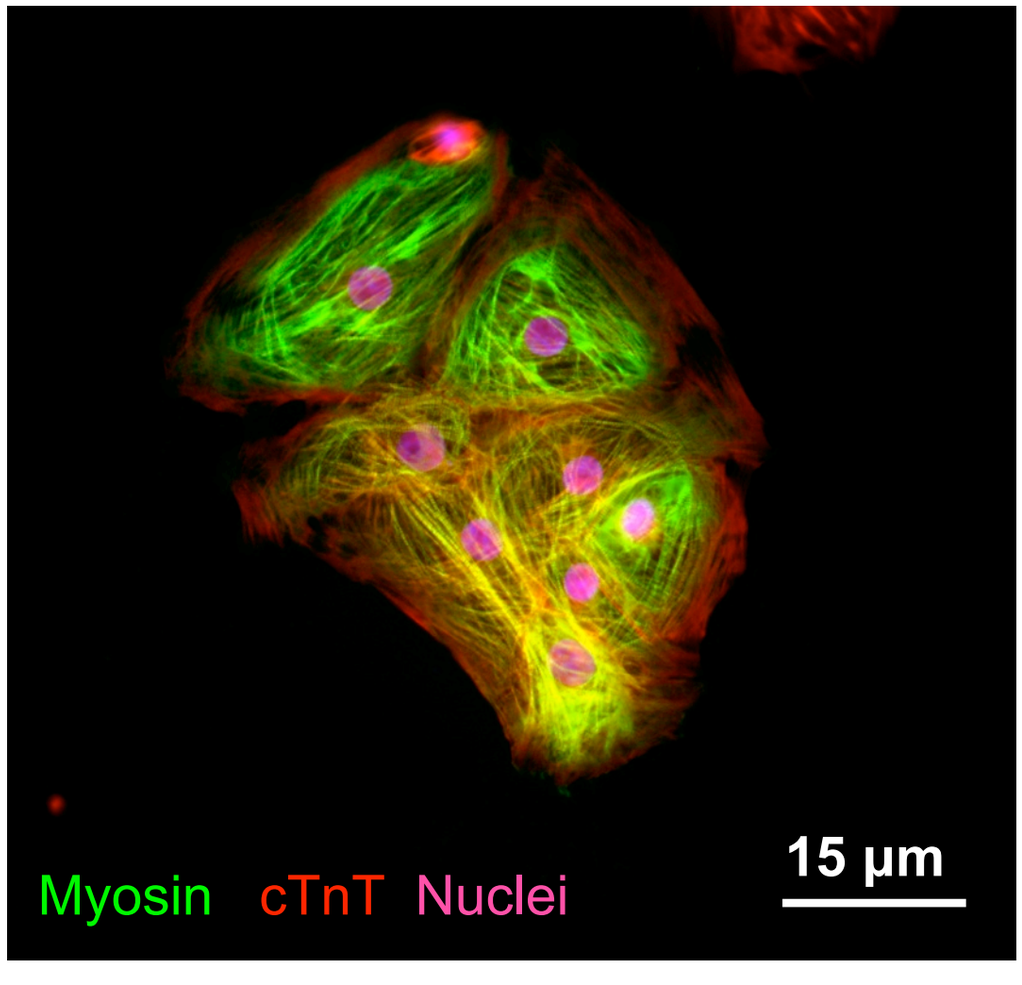


- Field potential duration (FPD)
- Beat rate

LQT3 hiPSC-CM derived Cardiac Tissue

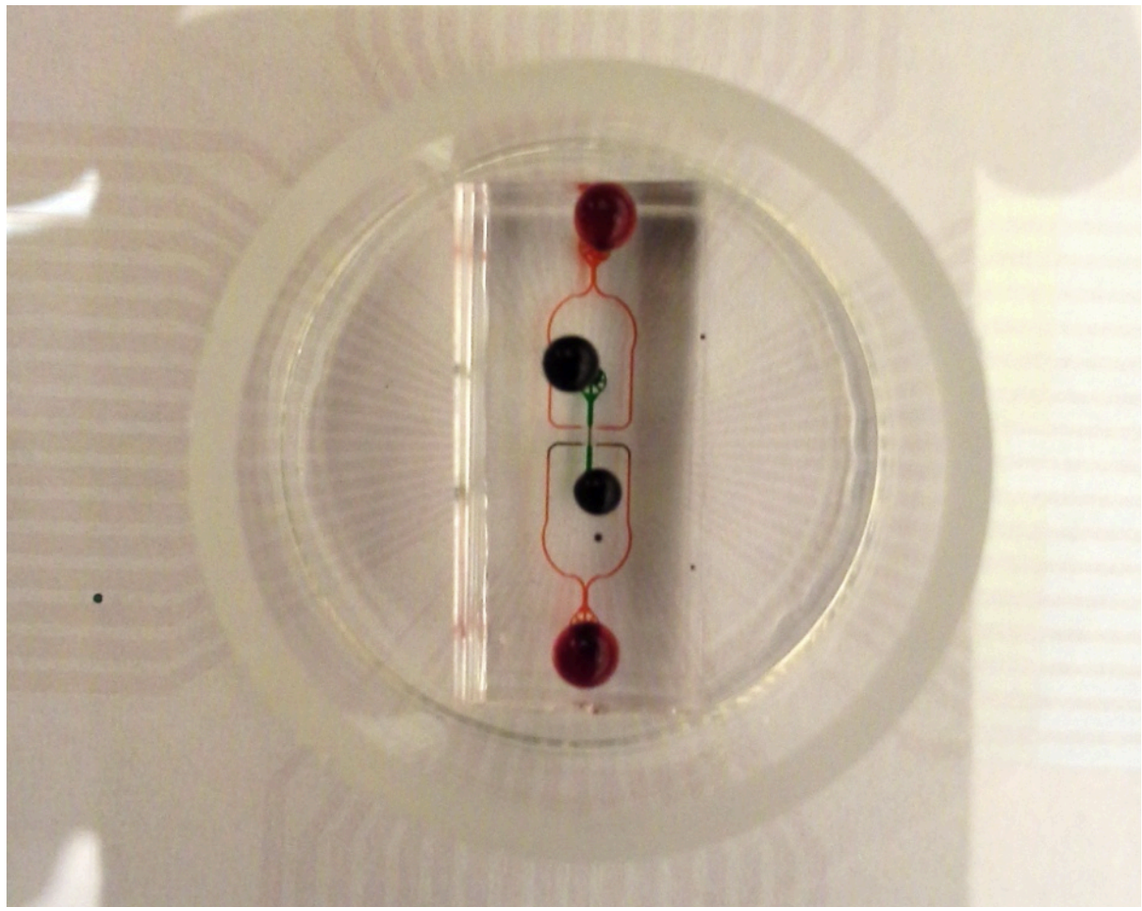


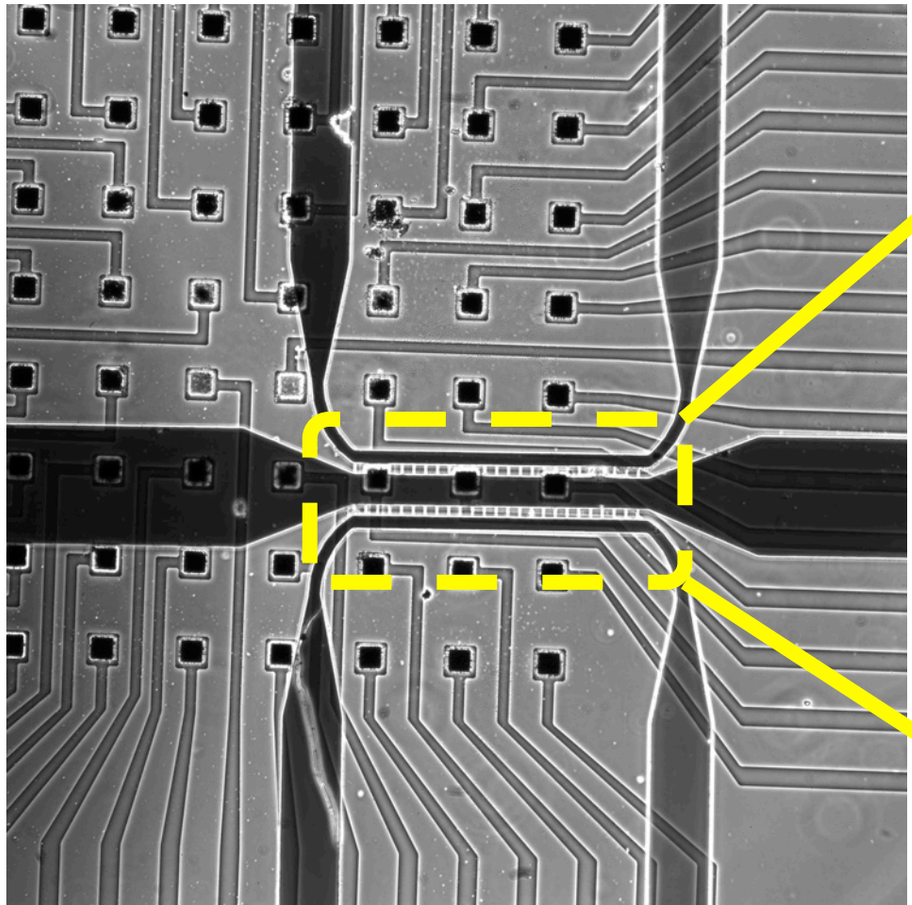


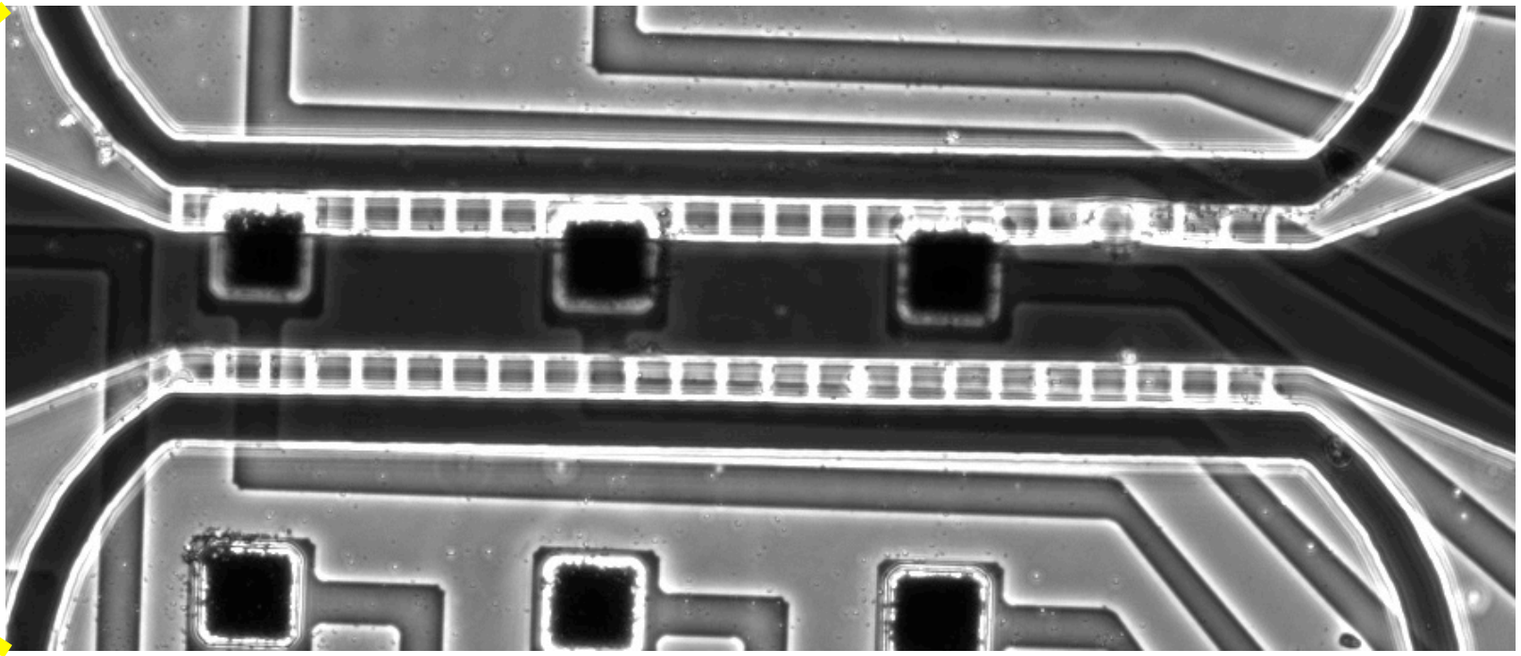


- E4031 blocks I_K
- Extends QT interval

Bonding of the Cardiac Chip to Commercial MEA

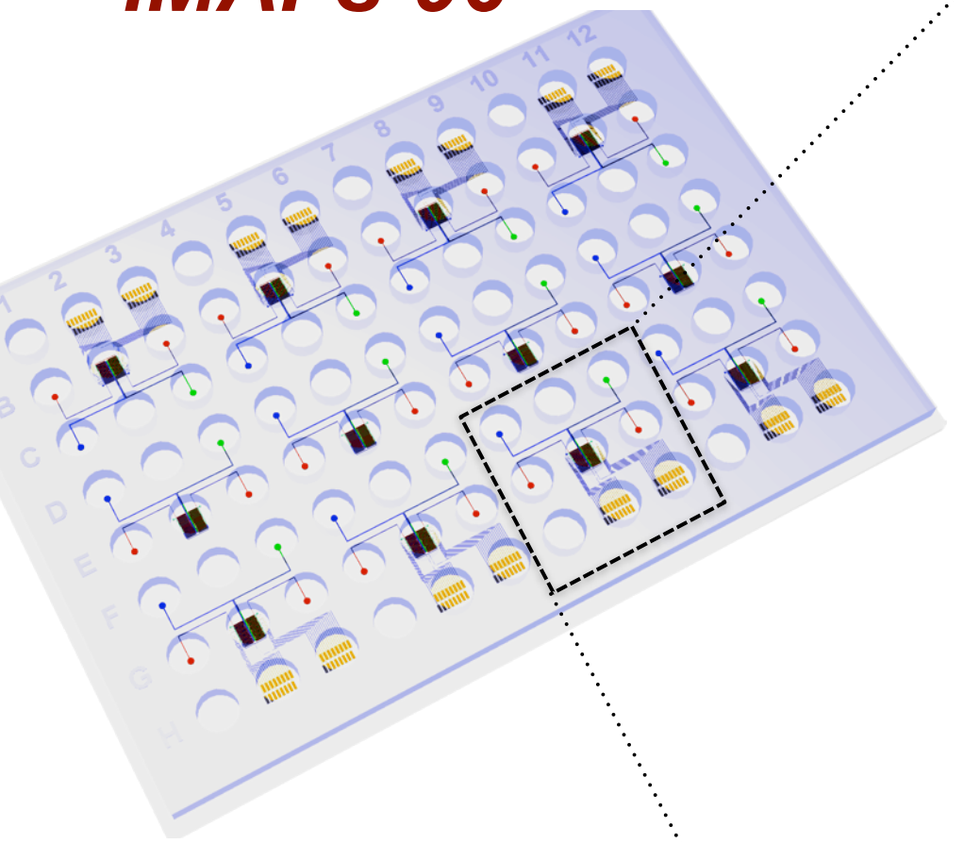


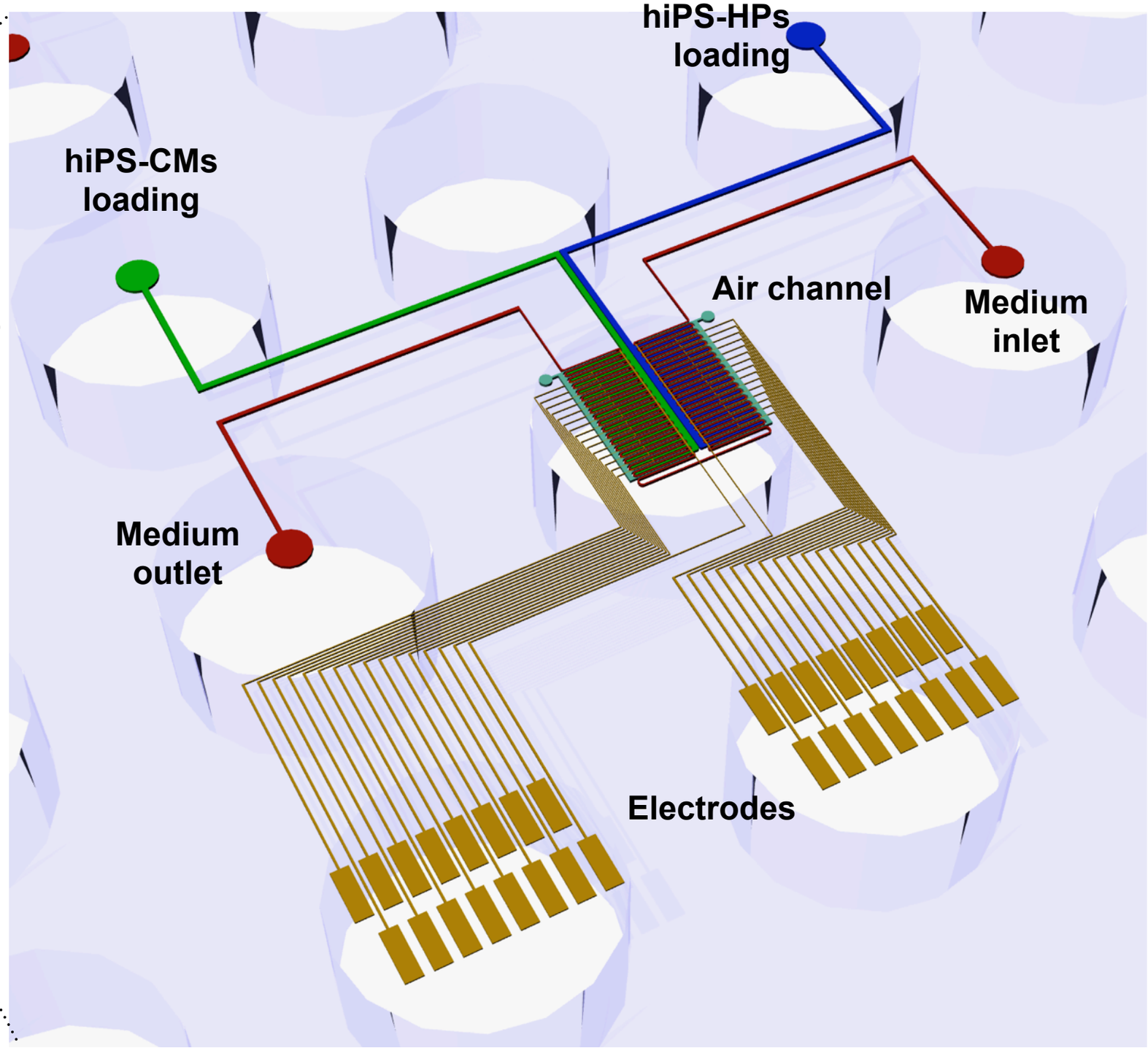




User-friendly "Tubeless" integrated Microphysiological Analysis Platform (*iMAP*) *on a 96-well plate*

iMAPs-96





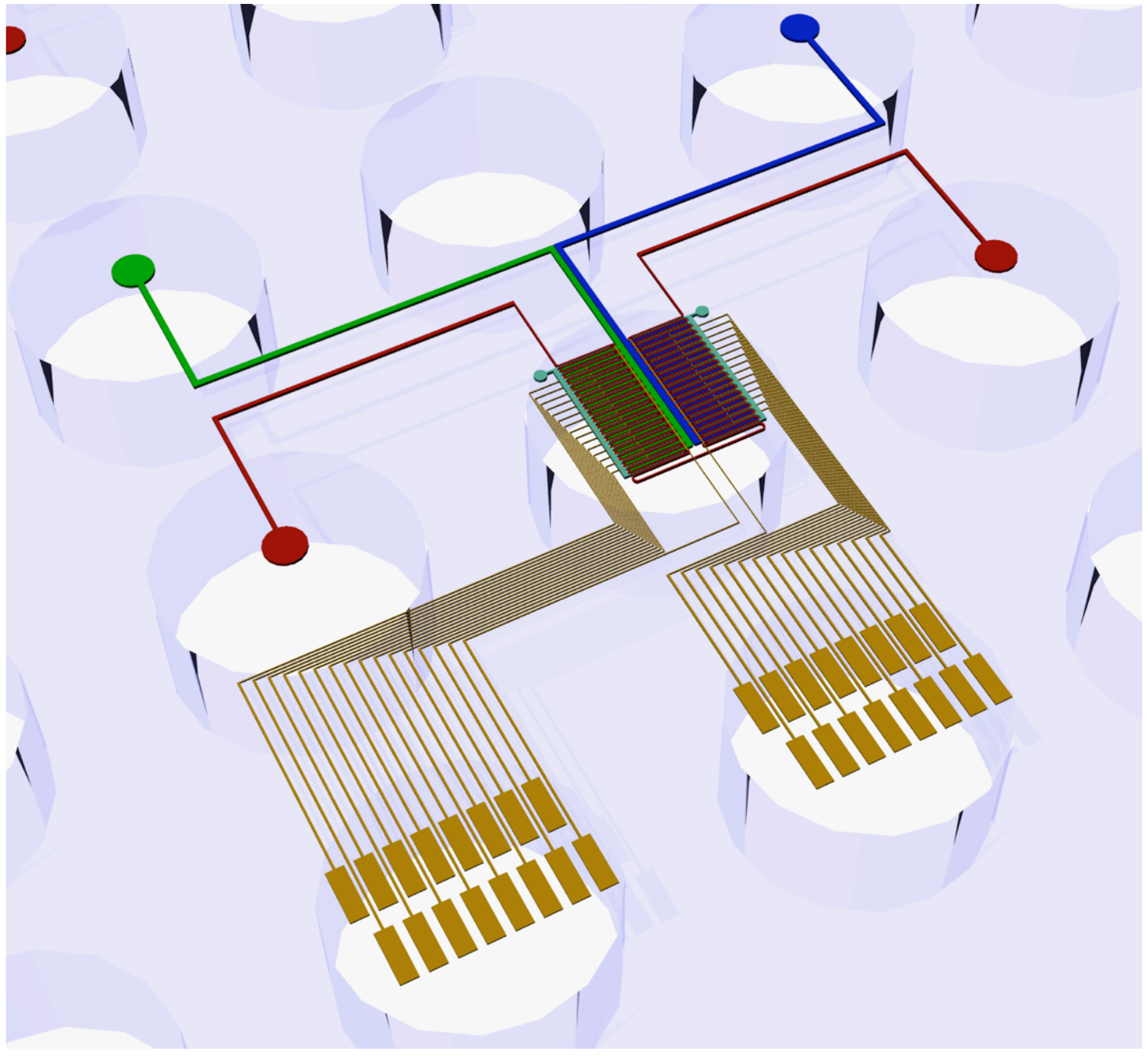
User-friendly "Tubeless" integrated Microphysiological Analysis Platform (*iMAP*) *on a 96-well plate*

Advantages

- Human & disease specific organoid
- Microfluidic endothelial-like cell barrier
- Continuous flow mass transport
- Low cost and ease of use
- Multiplexing
- Integrates with existing 96-well plate analytical platforms

Disadvantages

- Lacks interaction with vasculature
- Lacks Endothelial - HP or CM interaction



iMAPS Validation

iMAPS Liver

The activities of enzymes and transporters critical for hepatic drug metabolism will be at least 50% of those of hpHPs

Cytochrome P450 enzymes - I drug metabolism as well as general metabolism of the human liver: CYP1A1, CYP2B6, CYP2Cs, CYP2D6 and CYP3A4

Phase II (UGT and SULT) drug-metabolizing enzymes

Phase 0 uptake transporters (OATP1Bs, OATs, OCTs), and 2 phase III efflux transporters (P-gp and BCRP) will be measured.

iMAPS Heart

Physiologically relevant mean field potential duration and beat rates

Greater than 75% accuracy of healthy and diseased cardiac tissue models response to drugs known to affect cardiac beat frequency, contractility, and metabolism

Qualification of drugs known to affect cardiac physiology and toxicity

Thank You

This research was supported by
NIH-NCATS UH2NS080691, NIH-NHLBI R01HL096525, NIH-NHLBI R01HL108677, Siebel Stem Cell Institute, and California Institute for Regenerative Medicine (CIRM)

University of California, Berkeley
Dr. Anurag Mathur
Dr. Peter Loskill
Dr. SoonGweon Hong
Dr. Kyungsuk Yum
Dr. Zhen Ma
Natalie Marks
Micaela Finnegan

University of California, San Francisco
Prof. Holger Willenbring
Laure Dumont

San Francisco - Gladstone Inst. Cardiovascular Disease
Prof. Bruce Conklin
Dr. Ian Spencer